PHOTOTAXIS IN *CHLAMYDOMONAS REINHARDTII*

ROBERT L. STAVIS and RONA HIRSCHBERG

From the Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT

Parameters which distinguish phototaxis from random motility in *Chlamydomonas reinhardtii* have been defined with quantitative assays. The phototactic responses in photosynthetic, mixotrophic, and heterotrophic cultures were highest during exponential growth and declined rapidly as the cultures entered stationary phase. In contrast, random motility was relatively constant throughout growth. Phototaxis and motility also differ in their sensitivity to azide and antimycin A. Both of these drugs inhibited phototaxis within 5 min, but motility was unaffected for at least 30 min. Phototaxis and motility have different ion requirements. Optimum motility was observed in the presence of either Ca^{++} or Mg^{++}; phototaxis required Ca^{++} and either K^{+} or NH_4^{+}. Photosynthesis is not required for phototaxis, since phototaxis was not inhibited by dichlorophenyldimethyl urea, and a mutant lacking chlorophyll was phototactic.

INTRODUCTION

Tactic responses of microorganisms to chemicals or light have drawn the attention of biologists for nearly a century, both for their intrinsic interest and as simple models of behavior (3, 4, 10, 12, 16, 19, 23, 27). These responses probably occur in all motile cells and are important in a wide variety of biological processes involving directed movements of cells. The cellular apparatus which mediates the response must consist of components which function as a stimulus receptor, a mechanism for controlling cell movement, and a system which links these two. Thus the response system can be thought of as a receptor, a transducer, and an effector, each of which may be a complex, multicomponent system. An understanding of the mechanism of tactic responses depends on identification of these components and analysis of their interactions. Such studies have been initiated with a number of systems including those by Adler and his colleagues of *Escherichia coli* chemotaxis (1, 17).

Chlamydomonas reinhardtii is a phototactic green alga which is amenable to both biochemical and genetic analysis. Previous work has shown that the cells respond to light by swimming in paths oriented toward the stimulus without changing the speed of movement (13). Analysis of the action spectrum for phototaxis has shown that the maximum response occurs with blue light (28), but the photoreceptor pigment has not been identified. Quantitative studies on the effects of growth conditions have shown that cultures synchronized with light and dark cycles have a circadian rhythm in their phototactic response which is maintained when the culture is transferred to constant light or dark conditions (6). However, cells grown in a continuous culture turbidistat have a constant phototactic response (28). Other effects of media and growth conditions have been noted but not characterized (13, 21, 24).

We are investigating the molecular basis of phototaxis in *C. reinhardtii* and have developed quantitative assays for measuring both phototaxis

and random motility. In our initial investigation, we have defined parameters which differentiate the phototactic response from random motility. These processes have been analyzed with respect to growth conditions, ion requirements, and bioenergetics; in each case differences which distinguish phototaxis from random motility were observed. The age of cultures greatly influenced the phototactic behavior while having only slight effects on motility. The ion requirements for phototaxis were more complex and specific than for motility, and phototaxis could be inhibited by agents which did not immediately affect motility. These results should make a further analysis of the behavior of *C. reinhardtii* possible.

MATERIALS AND METHODS

Algal Strains and Culture Conditions

C. reinhardtii, strain 21 gr (no nutritional requirements, [+] mating type), was obtained from R. Sager. U3N, a mutant lacking chlorophyll (15), was obtained from K.-S. Chiang. Cultures were grown photosynthetically on minimal salts medium (30) at 25°C in gyrotory shakers. The medium contains: 1.70 mM sodium citrate, 0.037 mM $FeCl_3 \cdot 6\ H_2O$, 0.36 mM $CaCl_2$, 1.22 mM $MgSO_4$, 0.73 mM KH_2PO_4, 0.95 mM K_2HPO_4, 3.75 mM NH_4NO_3, and 1.0 ml/liter of trace salts solution. The trace salts solution contains 16.17 mM H_3BO_3, 3.47 mM $ZnSO_4$, 2.37 mM $MnSO_4$, 0.84 mM $CoCl_2$, 0.12 mM $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4\ H_2O$, and 0.25 mM $CuSO_4$.

Illumination (20 J/m² per s) was provided by Naturescent fluorescent lamps (Duro-Lite Lamps, Inc., Fairlawn, N.J.). For heterotrophic and mixotrophic growth, minimal medium was supplemented with sodium acetate at concentrations as specified for individual experiments. Strain 21 gr was grown photosynthetically unless otherwise specified. Strain U3N was grown heterotrophically in the dark. Cell densities were measured by optical density at 600 nm or by counting formaldehyde-killed cells in a hemocytometer. An optical density of 1.0 is equivalent to 5×10^6 cells/ml in our spectrophotometer.

Phototaxis Assay

The response of cells to light was measured with a device designed after the "phototaxigraph" of Lindes et al. (20) (Fig. 1). It was machined in aluminum and brass by the Matex Company (Palisades Park, N.J.). The apparatus fits into the cuvette chamber of a Zeiss PMQ-II spectrophotometer (Carl Zeiss, Inc., New York). The optical density of cells in the actinically lighted section of the cuvette is measured at 800 nm and recorded as a function of time with a Zeiss TE converter and a Heath EU-20B recorder (Heath/Schlumberger Scientific Instruments Div., Heath Co., Benton Harbor, Mich.). The optical density of cells in the cuvette follows Beer's Law. The monochromatic measuring light of 800 nm does not stimulate a phototactic response, and the actinic light does not interfere with the measurement of optical density.

Phototaxis assays were conducted in the following manner: an assay tube was filled with a suspension of cells and the open end was closed with a serum stopper. The tube was positioned in the phototaxis machine, and the entire apparatus was fitted into the spectrophotometer with the stimulus light off. The optical density was recorded for 10–30 s, and then the actinic light source was switched on. Responses were recorded for 3–10 min.

Motility Assay

Random motility of microorganisms is analogous to molecular diffusion and can be described quantitatively by a motility coefficient M which is determined experimentally with the equation used by Adler and Dahl (2):

$$\ln c/c^\circ = -\tfrac{1}{2} \ln (\pi Mt) - [x^2/(4Mt)],$$

where c° is the initial number of particles at the origin, and c is the concentration of particles at a point x cm away from the origin at time t. M has the dimensions of square centimeters per hour. A plot of ln c/c° vs. x^2 yields a straight line with a slope of $[-1/(4Mt)]$.

Microcap disposable micropipettes (100 μl) (Drummond Scientific Co., Broomall, Pa.) were used as assay tubes. For each assay a tube was filled to within 1 cm of the end with test solution. The filled end was then plugged with Parafilm. At the start of the experiment the remainder of the tube was filled with ^{14}C-labeled cells (5×10^4 to 2×10^5 cells, containing $1\text{–}5 \times 10^5$ cpm), and the tubes were incubated horizontally at room temperature in the dark. After 2 h (unless otherwise specified) the tubes were scribed at 1 cm intervals with a diamond-tipped pencil and broken into pieces. Each piece was transferred to a separate scintillation vial and counted in Aquasol (New England Nuclear, Boston, Mass.). The fraction of total cells in each piece was determined from the counts.

Cells were prepared for assay as follows: for each assay 3–5 ml of radioactively labeled cells were collected by centrifugation at 5,000 rpm for 5 min, washed once in medium to remove excess radioactive label, and suspended at a concentration of 5×10^6 to 2×10^7 cells/ml. This concentrated suspension was used to fill the assay tubes. Inhibitors were added to minimal medium at the concentrations specified. The

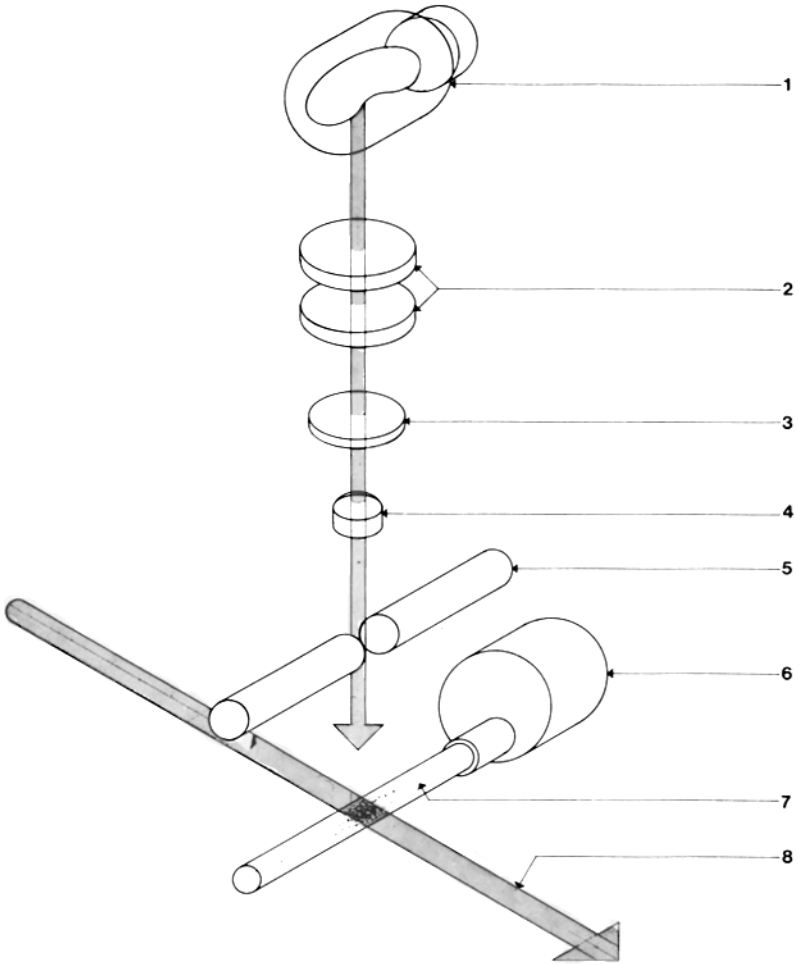

FIGURE 1 Phototaxis assay apparatus. The actinic light source (1) is a DFE 80 W projection lamp with an internal reflector. The lamp is run at 30 V AC, regulated by a Sola constant voltage transformer (Sola Basic Industries, Milwaukee, Wis.). The light passes through two heat filters (2), a ground glass disk (3), a collimating lens (4), and a variable width slit (5), and illuminates a 5 mm wide section of the assay cuvette containing the cells (7). A 10 rpm motor (6) rotates the cuvette around its longitudinal axis. The measuring light beam (8) passes through the actinically lighted region of the cuvette. The temperature is maintained at 25°C by water circulating through the body of the apparatus.The assay cuvettes are precision ground glass tubes with one closed end; they are 96 mm long, 9 mm OD, 7 mm ID, and have a volume of 3.5 ml.

immediate effect of inhibitors on motility was evaluated microscopically.

Preparation of ^{14}C-Labeled Cells

Photosynthetically growing cells were labeled with 2–5 μCi/ml of $NaH^{14}CO_3$ (sp act 55–60 mCi/mmol) for 2–4 h immediately before being harvested. Preparations with 0.5–2 cpm/cell were obtained. During mixotrophic and heterotrophic growth, cells were labeled with 5 μCi/ml of [1,2-^{14}C]acetate (Na salt) with the specific activities and concentrations as described for individual experiments. Cultures were labeled throughout growth, and preparations with 1–2 cpm/cell were routinely obtained.

CO_2 Incorporation

CO_2 fixation was measured titrimetrically (14) with a Radiometer TT-1 titrator (Radiometer A/S, Copenhagen). The amount of acid added to maintain a constant pH depends upon the amount of CO_2 taken up from the medium by the cells (25). Cells were collected by centrifugation, washed once

with deionized water, and suspended in an aqueous solution of 2 mM $NaHCO_3$ and 2 mM KCl at a concentration of about 10^7 cells/ml (equivalent to approximately 25 μg/ml of chlorophyll). The cell suspension was allowed to equilibrate at pH 7.5 in ambient light at 25°C for 5 min during which time the base-line rate of acid addition was recorded. The suspension was then illuminated with a 150 W flood lamp (82 J/m^2 per s) and addition of 0.1 M HCl was recorded as a function of time for about 30 min. The amount of acid added was taken to be equal to the amount of CO_2 incorporated. Inhibitors were added after 10 min illumination. Chlorophyll was determined by its absorbance at 665 and 649 nm in 80% acetone extracts (32).

Protein Synthesis

The assay measures incorporation of [^{14}C]arginine into cold TCA-insoluble material. Reaction mixtures containing 1.0×10^7 cells and 1.0 μCi of [^{14}C]arginine (sp act, 316 mCi/mmol) in 1.0 ml of minimal medium were incubated at 25°C in ambient light with gentle stirring. Samples of 0.1 ml were removed from the reactions and added to 0.5 ml of cold 10% TCA. The precipitated material was collected on Whatman GF/C glass fiber filters (H. Reeve Angel and Co., Inc., Clifton, N.J.), washed with four 5-ml portions of cold 5% TCA, dried, and counted in Aquasol.

Materials

Radioactive materials were obtained from Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N.Y. EDTA, EGTA, cycloheximide (CH), and 2,4-dinitrophenol (DNP) were purchased from Sigma Chemical Co., St. Louis, Mo. Dichlorophenyldimethyl urea (DCMU) was a gift from E. I. Dupont de Nemours and Co., Inc., Wilmington, Del. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) were gifts from W. Bertsch. Antimycin A was a gift from C. Moore. All other chemicals were reagent grade obtained from commercial sources. Phototaxis assay tubes were made by Wilmad Glass Co., Inc., Buena, N.J.

RESULTS

Phototaxis Assay

A typical phototaxis response curve is shown in Fig. 2. There was no response until the stimulus light was turned on (0 min). After a lag of 10–60 s the optical density of cells in the light path increased linearly for 1–3 min; the rate of accumulation then decreased until the OD reached a plateau. The maximum rate of increase, expressed as Δ OD per minute, is the rate of phototaxis. In this assay the rate was 0.45 OD/min.

The rate of phototaxis (Δ OD per minute) was a linear function of cell concentration (Fig. 3). Other experiments showed that the linear range extends from 0.01 to 0.6 OD. The rate can therefore be divided by the cell concentration (OD_{600}) to obtain a density-independent measure of the phototactic response. This normalized rate or phototaxis coefficient, "*PT*," expressed as $(\Delta\ OD_{800}/min)/OD_{600}$, makes it possible to compare phototactic responses at different cell densities. The *PT* of the culture shown in Fig. 3 was 0.62. Phototaxis assays were routinely carried out within the linear range of the assay. Reproducibility of assays is ±20%; differences greater than this are considered significant.

The assay measures the behavior of most of the cells in the population. When the plateau is reached (see Fig. 2), less than 15% of the total cells measured by optical density and less than 1% of the viable cells measured by plate counts remained in a sample withdrawn from the end of the assay cuvette.

Motility Assay

The phototaxis assay cannot discriminate between specific changes in phototaxis and changes in motility which would affect phototaxis. In order to do this a direct assay of motility is needed. Adler and Dahl's assay, modified as described, was used for this purpose.

Straight line plots of ln c/c° were obtained with cultures that appeared highly motile microscopically (Fig. 4, closed circles). *M* in this assay was 2.1. The plot of the data from the assay of the second culture (open circles) deviates from a straight line. Microscopic examination of this culture showed a nonhomogeneous population with up to one-third of the cells not swimming at any one time. In such cases *M* was calculated from the portion of the curve with the least slope, representing the fastest motility; in the assay shown *M* was equal to 1.5.

Individual cells had a velocity of 101 ± 16 μm/s when they were timed as they moved across a hemocytometer grid. In the tube assay, where cells were swimming randomly in three dimensions, a few cells traveled 100 mm in 2 h, a net forward velocity of 14 μm/s.

Theoretically *M* is independent of both the

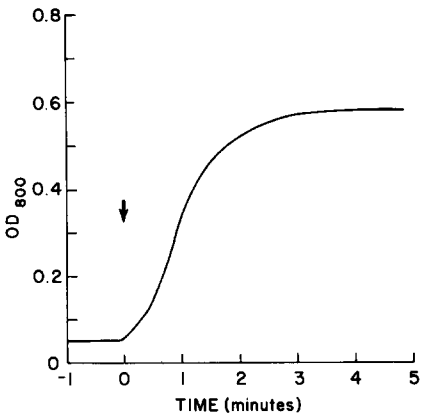

FIGURE 2 Tracing of a phototactic response recording. Photosynthetically grown cells were assayed during exponential growth as described in Methods. The stimulus light was turned on at the arrow.

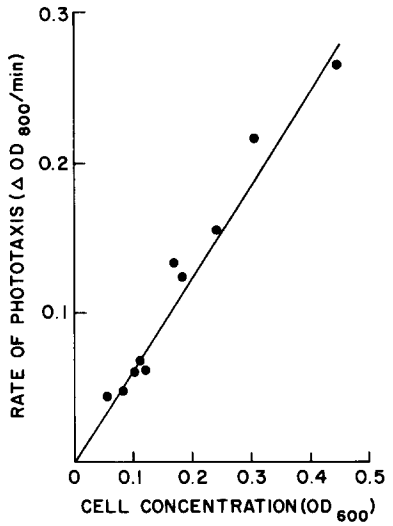

FIGURE 3 Concentration dependence of the phototactic response. Photosynthetically grown cells were harvested during exponential growth by centrifugation at 10,000 *g* for 5 min. Cells were suspended in the supernatant medium at the concentrations shown, and the rate of phototaxis at each concentration was determined.

initial cell concentration at the origin and the time period of the assay. Fig. 5 shows, as predicted, that M is not dependent on the number of cells introduced at the origin; M was 1.5 $\pm$ 0.1 cm^2/s over the 20-fold range of 2.5 $\times$ 10^4 to 5 $\times$ 10^5 cells. All subsequent assays were conducted within this range. M is not independent of assay time, however, and the values decrease as the assay period increases (Fig. 6). This reflects a fundamental difference between motility and diffusion. Thermal motion, which causes diffusion, is constant with time, whereas the amount of metabolic energy available for motility could be expected to decrease with time as cells deplete their energy reserves in the dark. The problem of variability due to length of assay period was controlled by running all the assays in an experiment for the same time, 2 h unless otherwise specified.

Motility coefficients of duplicate assays in the same experiment agreed within 15%. Variability in results from different experiments was somewhat greater. The effects of various agents or conditions on motility were, therefore, always measured with respect to control assays run at the same time.

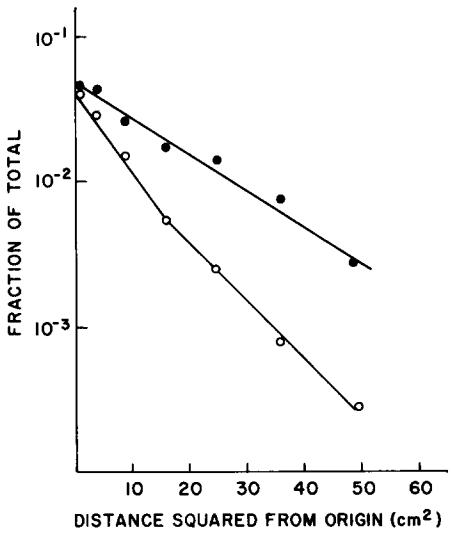

FIGURE 4 Plots of ln c/c° from two motility assays. Cultures were labeled with $NaH^{14}CO_3$, collected, and assayed as described in Methods. The open and closed circles represent the results from two different cultures.

Effect of Growth Conditions on Phototaxis and Motility

Exponentially growing photosynthetic cultures were used in the experiments described in the previous sections. Experiments were conducted to determine whether the phototactic response is affected when cultures are grown with acetate in the light and dark, or when cells are not growing exponentially.

Phototaxis and motility throughout the growth of a photosynthetic culture were examined (Fig. 7). At the times indicated, motility, OD_{600}, and PT

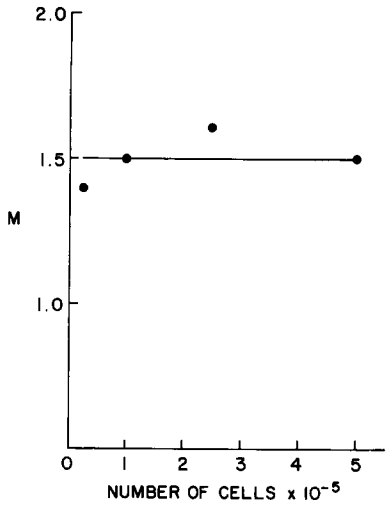

FIGURE 5 Dependence of motility on the number of cells at the origin. A labeled culture was harvested, and portions were adjusted to various densities, such that the number of cells shown was introduced at the origin of the assay tube in 8 μl. Motility coefficients (M) were determined from 2-h assays.

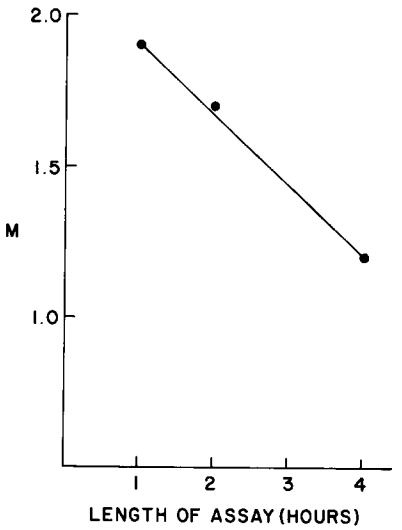

FIGURE 6 Dependence of motility on the length of the assay period. Motility coefficients (M) were determined in 1-, 2-, and 4-h assays.

were measured. There were two distinct phases of phototactic behavior which occur in the two phases of growth. During rapid exponential growth (24–48 h), the PT values were relatively constant and high, averaging 1.1. At the end of rapid growth (50 h), there was an abrupt decrease in the PT values to about 0.5, and during the slow growth

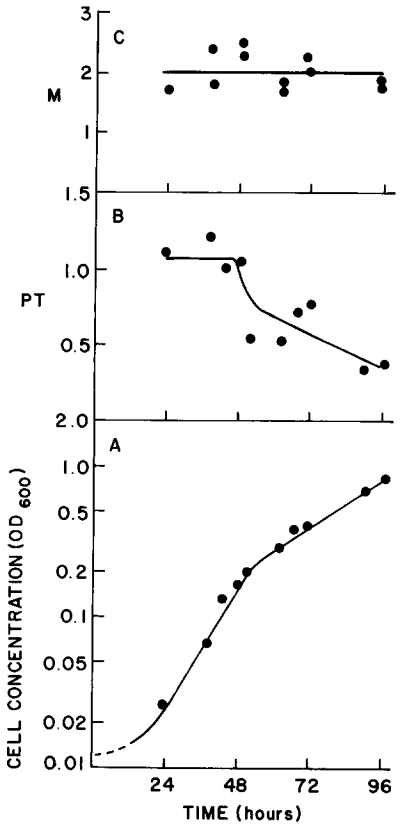

FIGURE 7 Phototaxis (PT) and motility (M) throughout growth of a photosynthetic culture. 2 h before each time point a 10-ml sample of the culture was labeled with $NaH^{14}CO_3$ in a parallel culture. At the times indicated growth (A), phototaxis (B), and motility (C) were measured.

phase (55–96 h), the values decreased even further. Part of the decrease during the last 18 h of growth can be attributed to the nonlinearity of the assay at these high cell densities. Motility was fairly constant, with M values averaging 2.0 throughout the experiment. Phototaxis, but not motility, varies with the age of the culture and is maximum during rapid balanced growth.

A similar experiment was conducted with a mixotrophic culture grown in the light on minimal medium supplemented with sodium acetate as the carbon source. At the times indicated, motility assays were started, and the OD_{600} and PT were measured. The results are similar to those of the previous experiment (Fig. 8). Again the PT values were high during rapid growth and decreased when growth slowed (50 h). A gradual decline in M values was observed in this culture during the

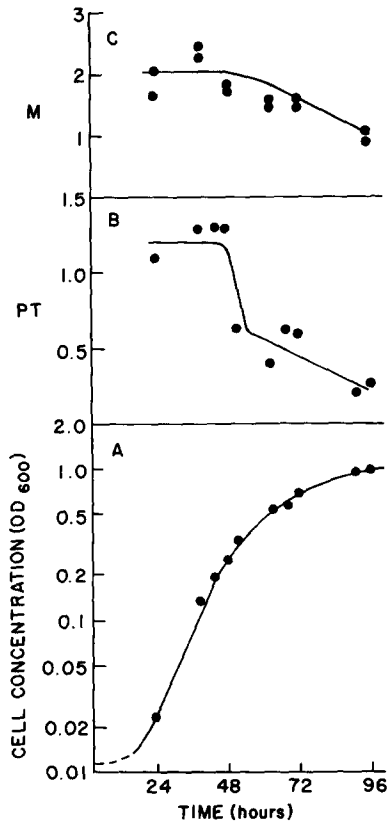

FIGURE 8 Phototaxis (*PT*) and motility (*M*) throughout growth of a mixotrophic culture. Minimal medium was supplemented with 0.1% sodium [^{14}C]acetate (5 μCi/ml). At the times indicated growth (A), phototaxis (B), and motility (C) were measured.

slow growth phase; some cells were in clumps, and many were missing one or both flagella. With a higher concentration of acetate (0.2%) there was a more pronounced and earlier effect on motility. The specific decline in phototaxis which occurred at the end of rapid exponential growth in photosynthetic cultures also occurred with acetate.

Cultures were grown heterotrophically in the dark with 0.1% sodium acetate as carbon and energy source. Under these conditions, growth was very slow (doubling time about 20 h). Since actively motile cultures were not always obtained, phototaxis varied from culture to culture. In those experiments with actively motile cells phototactic behavior was similar to that seen in light-grown cultures. *PT* values were high during exponential growth (*PT* = 0.8–1.2) and declined as the cultures entered stationary phase. In one experiment, a culture was maintained through eight doublings in the dark by transfer into fresh medium. During exponential growth of the subculture (six doublings in the dark) *PT* values of 1.4 were observed. This shows that phototaxis-specific cell components continue to be synthesized in the dark. Low phototaxis coefficients in dark cultures always correlated with poor motility and appeared to be a secondary effect resulting from the generally poor physiological state of the cells under these conditions.

Effects of Inhibitors on Phototaxis and Motility

Other aspects of the physiological requirements for phototaxis and motility were investigated by studying the effects of inhibitors on photosynthetically grown cells in exponential growth (Table I). Cycloheximide inhibits cytoplasmic protein synthesis (31) and, at a concentration of 10 μg/ml, inhibited [^{14}C]arginine incorporation by greater than 95% in 10 min. Apparently, cytoplasmic protein synthesis is not required for phototaxis and motility, since both were unaffected for up to 4 h in the presence of this drug.

Inhibitors of oxidative phosphorylation and photophosphorylation were also tested. Sodium azide (10^{-3} M) and antimycin A (10 μg/ml) immediately inhibited phototaxis but did not affect motility, evaluated microscopically, for at least 30 min. The uncouplers DNP (10^{-3} M), CCCP

TABLE I

Effect of Inhibitors on Phototaxis and Motility

Inhibitor	Concentration	Time	*PT**	*M**
Cycloheximide‡	10 μg/ml	4 h	105	100
DCMU§	1 μM	5 min‖	110	100
DNP	1 mM	5 min	0	0
CCCP	10 μM	5 min	0	0
FCCP	10 μM	5 min	0	0
Azide	1 mM	5 min	0	100
Antimycin A	10 μg/ml	5 min	40	100

* As percent of untreated controls.

‡ Incorporation of [^{14}C]arginine (sp act 316 mCi/mmol) was 4,433 cpm/10^6 cells per h in untreated cells and 96 cpm/10^6 cells per h 15 min after addition of CH.

§ Incorporation of $NaHCO_3$ was 1.98 nmol/μg chlorophyll per min in untreated cells and less than 0.04 nmol/μg chlorophyll per min 1 min after addition of DCMU.

‖ Motility at 5 min was evaluated microscopically.

(10^{-5} M), and FCCP (10^{-5} M) totally inhibited both motility and phototaxis, and microscopic examination of cultures treated with these drugs showed that all cells had lost their flagella. Whether this resulted from direct effects on ATP metabolism or was a secondary effect is not clear. Similar effects have been noted in other studies (5, 22). In either case, it is not possible to determine if these compounds have specific effects on phototaxis.

DCMU is a specific inhibitor of the Hill reaction in photosynthesis (18). At a concentration of 10^{-6} M DCMU, incorporation of CO_2 was totally inhibited but neither phototaxis nor motility was affected.

Phototaxis in U3N, a Mutant Lacking Chlorophyll

The lack of inhibition of phototaxis by DCMU strongly suggested that photosynthesis is not required for the response. This was confirmed by studying U3N, a mutant of *C. reinhardtii* which has no detectable chlorophyll and is consequently incapable of fixing CO_2 photosynthetically. Since U3N is photosensitive, it was grown heterotrophically in the dark on medium supplemented with 0.1% sodium acetate and 0.01% tryptone. Phototaxis was measured periodically as in the other growth studies. During exponential growth (OD_{600} = 0.1–0.2) *PT* values averaging 0.6 were observed. The fact that U3N is phototactic, despite its complete lack of chlorophyll, conclusively demonstrates that photosynthesis is not required for phototaxis.

Ion Requirements for Phototaxis and Motility

1 mM EDTA or 1 mM EGTA totally inhibited motility, suggesting a requirement for divalent cations. To characterize further the ion requirements of motility, labeled cells were collected by centrifugation, and then washed, suspended, and assayed in solutions containing different ions (Table II). In the absence of any added cations, motility was variable and average 44% of that observed in assays where the cells were suspended in the complete medium. We attribute the variability in motility when cells were assayed in buffer to incomplete removal of tightly bound ions during the washing procedure, since 0.1 mM EDTA completely inhibited motility in buffer. When either Mg^{++} or Ca^{++} was added to the buffer, the motility coefficients were equivalent to that observed in the control. The experiment also showed that neither K^+ nor Na^+ stimulated motility.

The ion dependence of motility is the same as that of the flagellar ATPase. Chelators completely inhibited ATP hydrolysis by whole flagella, and either Ca^{++} or Mg^{++} stimulated the ATPase activity two- to fourfold above the levels observed in buffer alone. It seems reasonable that the need for a divalent metal in motility is due to the requirement of the flagellar ATPase for these ions.

The ion requirements of phototaxis were also investigated (Table III). Exponentially growing cells were collected by centrifugation, washed once with 0.01 M Tris-HCl buffer, pH 7.0, then suspended in the solutions shown. When cells were suspended in buffer alone, the phototaxis coefficient

TABLE II

Ion Requirements for Motility

Assay solution	*M* (% of control)
Complete medium*	100
10 mM Tris-HCl, pH 7.0	44
+ 0.1 mM EDTA	5
+ 0.1 mM EGTA	0
+ 1 mM $CaCl_2$	98
+ 1 mM $MgCl_2$	87
+ 2 mM NaCl	43
+ 2 mM KCl	56

* Complete medium contains 1.7 mM sodium, 1.2 mM magnesium, 1.8 mM potassium, and 0.4 mM calcium.

TABLE III

Ion Requirements for Phototaxis

Assay solution	*PT* (% of control)
Complete medium	100
10 mM Tris-HCl, pH 7.0	5
+ NaCl*	3
+ KCl	13
+ $CaCl_2$	43
+ $MgCl_2$	3
+ NaCl, $CaCl_2$	29
+ NaCl, $MgCl_2$	3
+ KCl, $CaCl_2$	80
+ KCl, $MgCl_2$	3

* Each cation was tested at a concentration of 1 mM.

was 5% of that observed in complete medium. The addition of either Na^+, K^+, or Mg^{++} did not increase the response, but when Ca^{++} was added, the phototaxis coefficient was 43% of the control. Since no single ion gave a maximum response, combinations of monovalent and divalent cations were tested. In the presence of both Ca^{++} and K^+ the phototaxis coefficient was approximately equal to the control; Na^+ did not replace K^+ in combination with Ca^{++}, and Mg^{++} was not effective with either Na^+ or K^+. In other experiments it was shown that K^+ may be replaced by Cs^+, Rb^+, or NH_4^+, but that none of these monovalent cations alone were effective in stimulating phototaxis. Thus, it is clear that motility and phototaxis differ in their ion requirements.

DISCUSSION

Criteria have been developed by which phototaxis and motility may be differentiated. Studies of growth, effects of metabolic inhibitors, and ion requirements clearly show that although phototaxis requires motility, there are cell components and processes unique to phototaxis. This information should be useful in further studies of the mechanism of phototaxis.

The observation that azide and antimycin A inhibit phototaxis immediately but do not affect motility for at least half an hour is particularly interesting. Azide inhibits cytochrome *a* oxidation in mammalian mitochondria (34), but it is not clear that this site of inhibition is present in *C. reinhardtii*. In a pale green mutant the concentration of cytochrome a_3 was so low that it was questioned whether this cytochrome could function as the terminal oxidase (8). Antimycin A is known to inhibit electron transfer from cytochrome *b* to cytochrome c_1 (7), and it also inhibits cyclic photophosphorylation in chloroplasts (29). The effect of antimycin A on phototaxis is probably not related to its effects on photosynthesis, since phototaxis was observed in U3N, a strain in which no photophosphorylation can occur due to its lack of chlorophyll. If these drugs inhibit oxidative phosphorylation in *C. reinhardtii*, ATP synthesis would cease, but it appears unlikely that cellular ATP levels could have been grossly affected at the time when phototaxis was inhibited, since motility, which requires ATP, was unaffected. Other mitochondrial functions, such as ion transport and maintenance of electrochemical gradients might also be inhibited by these drugs and could be involved in phototaxis. Alternatively, the effects of these drugs may result from their interaction with some as yet unidentified phototaxis-specific cell components.

This study has also clarified the role of photosynthesis in phototaxis. Previous work on the action spectrum has shown that chlorophyll is not the phototactic receptor, but a metabolic role of photosynthesis was not ruled out. We have found that cells grown photosynthetically, mixotrophically, and heterotrophically were all phototactic, suggesting that synthesis of phototaxis-specific cell components does not depend on photosynthetic growth or even on growth in the light. Moreover, it appears that oxygen evolution, carbon dioxide fixation, and noncyclic photophosphorylation and electron transport are not required for phototaxis because DCMU did not inhibit phototaxis. In contrast, Diehn and Tollin reported inhibition of phototaxis in *Euglena gracilis* by DCMU, CCCP, and methyl octanoate and concluded that photosynthetic phosphorylation is the energy source for phototaxis (11). In view of the fact that inhibition was not observed until several hours after addition of the drugs, their results are subject to alternative interpretations. A definitive test of the role of photosynthesis was made with U3N, a mutant of *C. reinhardtii* which lacks chlorophyll. Gross and Dugger isolated this mutant and reported that it was phototactic (15). Their observation has been confirmed in this study, and we conclude that photosynthesis is not required for the synthesis of phototaxis components or for phototaxis itself in *C. reinhardtii*. This is different from the mechanism of phototaxis in some prokaryotic organisms, in which photosynthesis and phototaxis are thought to be coupled (9, 26, 33).

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