EFFECTS OF Mg^{2+} AND Ca^{2+} ON PHOTOINDUCED *EUGLENA* FLAGELLAR RESPONSES

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ABSTRACT

The flagellar frequency and waveform of *Euglena* were analyzed under full illumination (420–700 nm) and in a restricted wavelength band (530–700 nm) when the cells were in a medium containing Mg^{2+} or had been microinjected with Mg^{2+}, Mn^{2+}, or Ca^{2+} in solution. Magnesium abolished the change in flagellar frequency and the reversal in waveform that cells exhibit when illuminated by a 530–700-nm wavelength band. Under this restricted illumination, Ca^{2+} caused an increase in flagellar waveform reversal and a decrease in beating frequency. The flagellar motility of cells impaled on a microelectrode was examined in cells illuminated with various wavelengths.

KEY WORDS *Euglena* · motility · photoinduction · magnesium

Euglena cell possess a primitive photoreceptor-effector. It is generally accepted that the stigma-paraflagellar swelling within *Euglena* captures light information, which is then translated via an as yet unknown mechanism into a flagellar response (5, 16, 17, 35).

The flagella of *Euglena*, when illuminated with light, show a helical, asymmetric wave motion (13), which is somewhat irregular in frequency. In this normal or forward waveform, the flagellum is extended and appears very flexible. Cells illuminated by a 530–700-nm wavelength band exhibit a change in waveform, a "reversal," and a change in beat frequency from normal (12, 16).

Previous experiments in this laboratory have shown that the flagellar motility of both *Euglena* and *Chlamydomonas* can be controlled with the application of electric current to the inside of the cells. This electrical control of flagellar frequency was Mg^{2+} but not Ca^{2+} dependent. Ca^{2+} did, however, cause the ejection of the flagellum upon impalement of the cell with a microelectrode (22).

In other experiments, Ca^{2+} has been shown to be involved in the control of the frequency of beating of mussel gill cilia (18, 19, 27, 31, 32). The control of the waveform of *Paramecium* cilia (20, 29), *Chlamydomonas* flagella (15, 28), and *Crithidia* flagella (14) has been shown to be Ca^{2+} dependent.

In this paper, experiments are described to elucidate the role of Mg^{2+} and Ca^{2+} in the light-induced control of flagellar motion in *Euglena*. Preliminary reports of portions of the material in this paper have appeared recently (21, 25).

MATERIALS AND METHODS

Organisms and Materials

Euglena gracilis, Pringsheim strain, was obtained from the American Type Tissue Culture Co., Rockville, Md. The cells were grown and maintained under constant illumination at 21°C in a modified Cramer-Meyers medium (8) containing 7.3 mM KH_2PO_4, 7.5 mM $(NH_4)_3PO_4$, 1.7 mM $MgSO_4$, 0.2 mM $CaCl_2$, and trace metals.

Experiments were performed with <0.05 ml of *Euglena* culture in 1 ml of a minimal medium (MM) containing 7.3 mM KH_2PO_4 and 7.5 mM $(NH_4)_3PO_4$ in glass double-distilled H_2O, pH 7.0. The normal motility of the cells was maintained for up to 2 h in this medium.

ATP, disodium salt, was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Solutions of 0.1 M ATP at pH 7.0 were frozen in aliquots of 1 ml. For experiments, additions of ATP were made from an aliquot thawed just before use. EGTA, adjusted to pH 7.0 for use, was obtained from Sigma Chemical Co., St. Louis, Mo.

For observations on live *Euglena*, the cells were suspended in MM only. If the *Euglena* were to be impaled, 1.5 mM EGTA was added to the MM. The addition of EGTA had been found to be necessary in earlier experiments (22). Without it the *Euglena* flagellum was ejected from the cell upon penetration of the pellicle by the microelectrode. The junction between an impaling electrode and the *Euglena* membrane is not tight (22), and, as a consequence, the ATP in the impaled cell diffuses out into the medium. To maintain the flagellar activity of impaled *Euglena*, it was necessary to add 10 mM ATP to the medium, as previously described (22).

Additions to the MM were made with a Hamilton syringe, Hamilton Co., Reno, Nev. The experiments were performed at 21 ± 1°C.

Apparatus

During experimentation, cells were viewed in a Zeiss Universal microscope with a 40 × Zeiss water-immersion objective. The apparatus for impaling and positioning the cells and the details concerning the methods for preparing microelectrodes and suction pipets have been described previously (22).

For microinjection experiments, a microelectrode filled with 1 M KCl; with 1 M KCl and 0.1 M $MgSO_4$ or $MnSO_4$; or with 1 M KCl and 0.02 M $CaCl_2$ and mounted in a piezoelectric driver, was connected with Intramedic polyethylene capillary tubing (Clay-Adams Co., Parsippany, N. J.) to a glass chamber, which, in turn, was connected with tubing to a 1-μl Hamilton syringe securely mounted in an aluminum block. A micrometer wheel fixed on the aluminum block advanced the plunger of the syringe to deliver a volume of liquid. The syringe, all plastic tubing, and the glass connector were filled with Versilube-F-50 silicon oil (General Electric Co., Silicon Products Dept., Waterford, N. Y.) to prevent evaporation of the electrolyte in the microelectrode and to prevent air bubbles in the tubing. Turning the micrometer wheel delivered a volume of oil from the syringe into the tubing-connector-tubing system and delivered a volume of solution from the microelectrode into an impaled cell (24). The method used to calibrate the specific volume mechanically microinjected into a cell has been described previously (24).

Experimental preparations were illuminated under darkfield as previously described (22). A 1-kW xenon arc light (Hanovia Lamp Div., Canrad-Hanovia, Inc., Newark, N. J.), with the wavelength band restricted to 420–700 nm with 3-mm GG420 and 4-mm KG3 glass filters (Schott Gen., Mainz, W. Germany), provided the illumination for the microscopy. Fig. 1 shows the light intensity as a function of wavelength in the band 420–700 nm incident on the preparation. The values in Fig. 1 were calculated from the data for emission of the xenon arc lamp and the transmission of the GG420 and KG3 filters provided by the manufacturers. It can be seen in Fig. 1 that, in the 430–650-nm wavelength range, intensity is nearly independent of wavelength.

The wavelength band was restricted by positioning sharp cutoff filters of the GG, OG, or RG series (Schott Gen.) in the light path. In Fig. 1 are shown the light intensities *vs.* the wavelengths thus obtained. In the remainder of this paper, the limits of a wavelength band indicate the wavelength at which the transmitted light was reduced to 50%. Narrow-band illumination was obtained by appropriate combinations of the interference filters 90-1-620 and 90-4-500 of Bausch & Lomb Inc., Scientific Optical Products Div., Rochester, N. Y. and the glass filters mentioned above. Fig. 12 *C* shows the bands thus composed.

Two narrow bands of illuminating light were mixed by positioning filters on a glass support in the light beam close to the entrance of the microscope condenser (Fig. 2). By shifting the position of the glass support, the ratio of the amount of light reaching the preparation in band I to that in band II could easily be changed.

Wolken has reported (34) that the maximal photokinetic effect on *Euglena* is caused by light with a wavelength of <530 nm. In all the experiments, except those described in Effects of Various Wavelengths, the photo effects were induced by a low wavelength cutoff at 530 nm.

Cinemicrography

Cinemicrographs were made on Kodak 16-mm Plus X film at 200 frames/s with a Millikan PBM5C camera (Teledyne Camera Systems, Arcadia, Calif.). With restricted wavelength bands, the amount of illuminating light was decreased, and the quality of the moving films deteriorated. No filming was possible when the wavelength cutoff was at 610 nm or higher, or when the narrow-band illumination (Fig. 12 *C*) was employed.

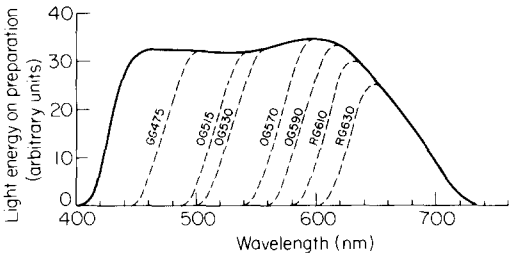

FIGURE 1 Light energy as a function of wavelength used in the experiments. The solid line indicates the value for full illumination (420–700 nm). The dotted lines indicate the low wavelength cutoff values for the various glass filters used.

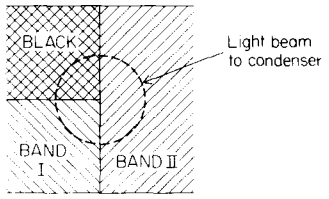

FIGURE 2 Diagram illustrating the way in which filter combinations isolated the narrow spectral bands shown in Fig. 12.

Motility Analysis

Films were projected to a final magnification of 1,000 in a Vanguard Motion Analyzer (Vanguard Co., Melville, N. Y.). The outline of the flagellum and a portion of the cell from successive frames were traced on transparent paper. The flagellar frequency was obtained from the period between repeating shapes as described previously (22).

For a detailed analysis of the waveforms of the *Euglena*, the 16-mm films were scanned, and four cells representing either the characteristic forward or the reversed flagellar waveforms were chosen for analysis. The cells were rephotographed on 35-mm film and projected to a final magnification of 5,000. Successive frames of the enlarged image were then traced. The individual tracings were overlaid to obtain an envelope of the complete flagellar pattern, and a line was drawn through the center of the envelope to represent a median reference axis. Distances of 2 μm were marked off along the length of the flagellum on each tracing to define the running coordinate S. Deviations from the median axis at various values of S were measured and plotted as a function of time, yielding the amplitude of the flagellar motion as a function of S (22, 26).

The curvature of the *Euglena* flagella was measured as illustrated in Fig. 3. Tangents were drawn to the tracing at, e.g., $S = 4$ μm and $S = 6$ μm. The curvature $\partial\theta/\partial S$ at $S = 5$ μm was then approximated as $\Delta\theta/\Delta S$. It has previously been estimated that the accuracy in $\partial\theta/\partial S$ thus obtained is $\pm$ 200 cm^{-1} (26). Deviations from the median axis at $S = 0$–15 μm were measured and plotted as a function of time. This method of measurement, illustrated in Fig. 3, has been used previously to analyze flagellar amplitudes of *Euglena* (22) and the movement of sea urchin sperm flagella (26).

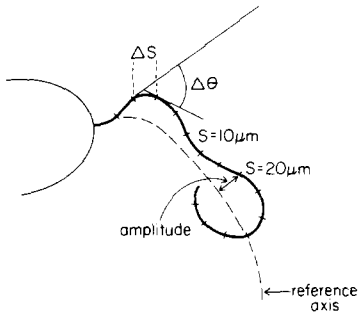

FIGURE 3 Illustration of the method used to measure the amplitude and the curvature of the flagella. For further explanation, see Motility Analysis.

The line width of the tracings was kept to ~0.5 mm by careful drawing. Tangents to the tracings were drawn as judged by eye to an accuracy of 1.5°. Measurements paralleling this accuracy have been made previously in sea urchin sperm (26).

The *Euglena* flagellar waveform is helical (13, 16, 17). Thus, as a result of projection errors and foreshortening, appreciable errors can be present in the amplitude, the curvature, and the value of S. However, because we obtained regular patterns for the amplitudes and curvatures, we believe that our measurements are meaningful.

RESULTS

Initial Observations

Free-swimming *Euglena* in MM with no additions exhibited normal forward flagellar motility when cells were illuminated by a 420–700-nm wavelength band. When the wavelength was restricted to 530–700 nm by means of an OG530 filter, flagellar wave motion changed to a reversed pattern, to be described in detail below. Fig. 4 shows tracings of the normal end of the reversed flagellar wave motion at 5-ms intervals. In the reversed motion, the proximal portion of the flagellum is bent backward, resulting in a changed direction of motion in the distal part of the flagellum.

Free-swimming cells illumianted in a restricted wavelength band of 530–700 nm did not maintain a reversed waveform pattern for longer than 0.5–1 min. After the reversed flagellar motion had caused a reorientation of the cell body, the flagellar waveform most often reverted to the normal forward pattern. This is reminiscent of the flagellar reversal in free-swimming cells, which was characterized by Engelmann (11) as "shock movement." He observed that, as *Euglena* passed from a lighted area to a shaded area, the flagellar waveform changed, which caused the cell to swim backward for a short distance. The cell then resumed forward motion in a different direction.

After *Euglena* had been impaled by a micro-

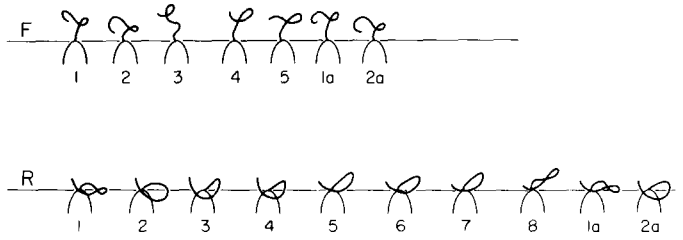

FIGURE 4 Tracings of the flagellum of free-swimming *Euglena* in MM at 5-ms intervals with normal, forward waveform (*F*) and with reversed waveform (*R*). Forward waveforms were traced from cells illuminated by a 420–700-nm wavelength band; reversed waveforms were under 530–700 nm light.

electrode, the flagellar reversal induced by restricting the illuminating light to a 530–720-nm wavelength band was maintained for a long period. The *Euglena* in these experiments were suspended in MM to which 1.5 mM EGTA and 10 mM ATP but no Mg^{2+} had been added.

Under full illumination, the impaled *Euglena* had a flagellar frequency of 15 ± 3 Hz, and 8% of the cells showed a reversed flagellar waveform (average and standard deviation of 12 cells). Several seconds after the insertion of the OG530 filter, 80% of the cells displayed reversed flagellar waveform, and the flagellar frequency had decreased to 7 ± 5 Hz. Fig. 5 shows that, during an 8-min period after the insertion of the OG530 filter, the *Euglena* flagella remained 80–90% reversed, and flagellar frequency gradually dropped. In the first several minutes after the insertion of the filter into the light path, steady-state measurements were possible (Fig. 5).

Waveform Analysis

For detailed analysis of the flagellar waveforms, live *Euglena* in MM were filmed under full illumination (420–700 nm). For the reversed motion, cells were filmed within 30 s after insertion of an OG530 filter, which restricted the illuminating light to a 530–700-nm band. Two normal and two reversed cells that showed no translational motion of the cell body during the time period in question were selected for detailed analysis. It proved to be impossible to obtain meaningful data on the curvature of the flagella when the cells had translational motion.

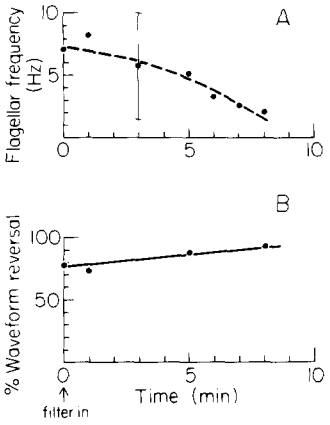

FIGURE 5 Flagellar frequency (*A*) and percent waveform reversal (*B*) of impaled *Euglena* as a function of the time elapsed after the insertion of the OG530 filter. Each point represents an average of 10–15 cells. The vertical bar in this figure and in Figs. 8–12 represents a typical standard deviation for the flagellar frequency. The standard errors for each point are approximately three times smaller than the standard deviation. The lines in Figures 5–12 are heuristic.

Fig. 6 shows the amplitude for forward and reversed motion of the flagellar waves as a function of location on the flagellum. Especially in the distal portion of the flagellum ($S > 10$ μm), the amplitude in the reversed motion is severely reduced. This confirmed the impression obtained from visual observation of the reversed waveforms.

Fig. 7 shows the variation of the curvature vs.

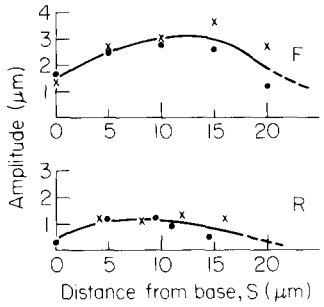

FIGURE 6 Average amplitude on various locations of free-swimming *Euglena* flagella. Forward waveforms (*F*) were exhibited in a 420–700-nm wavelength band. Cells with reversed waveforms (*R*) were illuminated by a 530–700-nm wavelength band. × and ● represent two cells from which data were obtained.

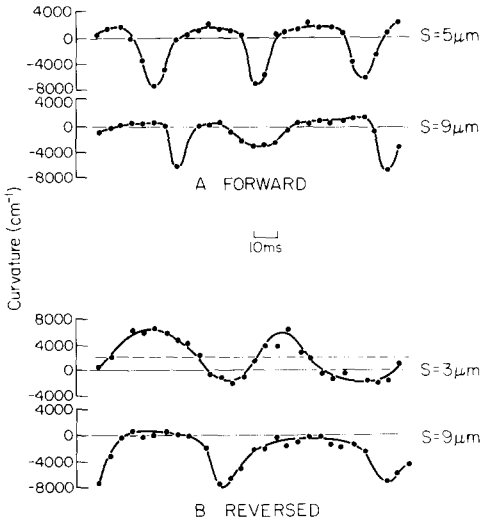

FIGURE 7 Curvature at various locations (*S*) on the flagellum of a *Euglena* with a forward waveform (*A*) and a reversed waveform (*B*).

time measured close to the point of insertion and at a more distal location on the flagellum of a normal and a reversed *Euglena*. In the forward motion, the flagellum at $S = 5$ μm is largely straight, with the curvature resulting predominately on one side. As Fig. 7*A* illustrates, for $S = 9$ μm, this pattern was seen also in the more distal parts of the flagellum.

In the reversed mode of flagellar motion, the proximal part of the flagellum has a more or less harmonic variation of the curvature (Fig. 7*B*). The average curvature is offset from zero by ~2,200 cm^{-1}. This corresponds to a radius of curvature of ~4.5 μm. The distal part of the flagellum, $S > 8$ μm, displayed a variation in curvature (Fig. 7*B*) similar to that observed in the forward motion.

The data in Fig. 7*B* confirm tht the mechanism of reversed flagellar motion in *Euglena* involves the bending backwards of the proximal section of the flagellar shaft, with a resulting reorientation of the distal part of the flagellum. It should be noted that in all cases (normal as well as reversed motion) the wave propagation was toward the distal tip of the flagellum.

Effects of External Mg^{2+}

Euglena cells in MM with 1.5 mM EGTA, 10 mM ATP, and various amounts of Mg^{2+} were impaled under constant illumination in a 420–700-nm wavelength band. Only those cells that exhibited normal forward flagellar waveforms upon impalement (~90%) were used for further experimentation.

When the wavelength band of the illuminating light was restricted to 530–700 nm by inserting an OG530 filter in the light path, all impaled cells contracted slightly within 50 ms after the filter was in place. The OG530 filter could be placed in the light path for 1 min and then removed for 1 min an average of 8 times, and the impaled cell would respond to the filter. Data were recorded for the experiments described in this section on the second filter pass, ~30 s after the insertion of the filter when a new steady state of flagellar beating had been reached.

Fig. 8 shows the percent reversal of flagellar waveform and the flagellar frequency as a function of the external Mg^{2+} concentration before and during the second insertion of the OG530 filter. It can be seen in Fig. 8 that, at an external Mg^{2+} concentration of 4 mM or more, the response of the *Euglena* flagellar wave motion to the restriction

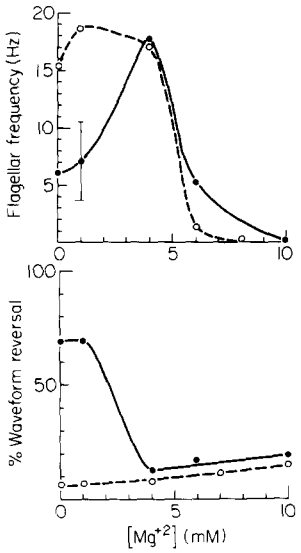

FIGURE 8 Flagellar frequency and percent waveform reversal of *Euglena* as a function of the external Mg^{+2} concentration. Impaled cells were in MM containing 1.5 mM EGTA and 10 mM ATP. 22 cells were filmed for the experiment. ○, cells illuminated by a 420–700-nm wavelength band. ●, cells illuminated by a 530–700-nm wavelength band.

of the wavelength band of the illuminating light is largely abolished.

Mechanical Microinjection of KCl, $MgSO_4$, $MnSO_4$, and $CaCl_2$

CONTROL EXPERIMENTS: The volumes microinjected into the *Euglena* in the experiments reported below ranged up to 30×10^{-14} liter. This is not a negligible fraction of the *Euglena* cell volume of $\sim 300 \times 10^{-14}$ liter. To test the effects of the internal application of specific volumes of an ionic solution, *Euglena* in MM with 1.5 mM EGTA and 10 mM ATP added were impaled with a microelectrode filled with 1 M KCl. Flagellar frequency, which was 14 ± 3 HZ in a 420–700-nm wavelength band, decreased to 6.5 ± 5 Hz with the restriction of the wavelength band to 530–700 nm (Fig. 9). Under this illumination, 78% of the impaled cells exhibited flagellar reversal. With the OG530 filter constantly in the light path, the injection of from 2 to 13×10^{-14} liter of 1 M KCl did not significantly alter the flagellar beating. The injection of $>14 \times 10^{-14}$ liter of 1 M KCl caused a decrease in flagellar frequency, possibly through mechanical damage to the cells.

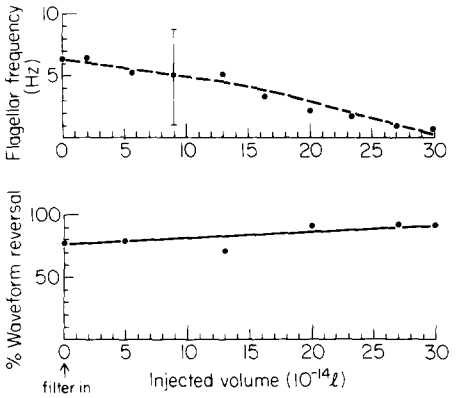

FIGURE 9 Flagellar frequency and percent waveform reversal vs. the volume of 1 M KCl injected (average of 12 cells). In this figure and in Figs. 10 and 11, *Euglena* were in MM with 1.5 mM EGTA and 10 mM ATP. Cells were under constant illumination by a 530–700-nm wavelength band.

INJECTION OF MG^{+2} AND MN^{+2}: *Euglena* in MM containing 1.5 mM EGTA and 10 mM ATP were impaled with a microelectrode filled with 1 M KCl and either 0.1 M $MgSO_4$ or 0.1 M $MnSO_4$. The flagellar frequency of the cells under full illumination (420–700 nm) was 15 ± 4 Hz. When the wavelength band was restricted to 530–700 nm with the OG530 filter, the flagellar frequency decreased to ~6 Hz, and 80% of the flagella were reversed. The injection of from 2 to 15 × 10^{-14} liter of either 0.1 M $MgSO_4$ or 0.1 M $MnSO_4$ caused a sharp increase in flagellar frequency of 15–18 Hz, and flagellar reversal decreased to ~10–20% (Fig. 10). The injection of >20 × 10^{-14} liter of either Mg^{2+} or Mn^{2+} resulted in smaller effects on the flagellar activity, as Fig. 10 shows, but because, at these large injected volumes, mechanical damage may have been caused to the cells, we have not attached much significance to this observation.

MICROINJECTION OF CA^{+2}: The flagellar frequency of *Euglena* in MM containing 1.5 mM EGTA and 10 mM ATP and impaled with a microelectrode filled with 0.02 M $CaCl_2$ and 1 M KCl decreased from 15 ± 4 Hz, under full illumination, (420–700 nm) to 6 ± 4 Hz under restricted illumination (530–700 nm), with 86% of the cells in flagellar reversal. The microinjection of 0.02 M Ca^{2+} caused a further decrease in flagellar activity (Fig. 11*A*). Complete flagellar arrest occurred at injected volumes of >13 × 10^{-14} liter. For all injected volumes of 0.02 M Ca^{2+}, the flagella remained in reversed waveform (Fig. 11*A*). When injected with <3 × 10^{-14} liter, cells recovered to pre-injection flagellar motility within 1 min. The injection of greater volumes of solution caused flagellar frequencies to remain decreased.

Recently, we reported the effects of microinjection of Ca^{2+} on the flagellar frequency of impaled *Euglena* under full wavelength band (420–700 nm) illumination (24), but we did not examine waveform patterns. We have now reexamined the cinemicrographs of those experiments for the occurrence of reversed waveforms after the injection of Ca^{2+}. Figure 11*B* shows that, under full illu-

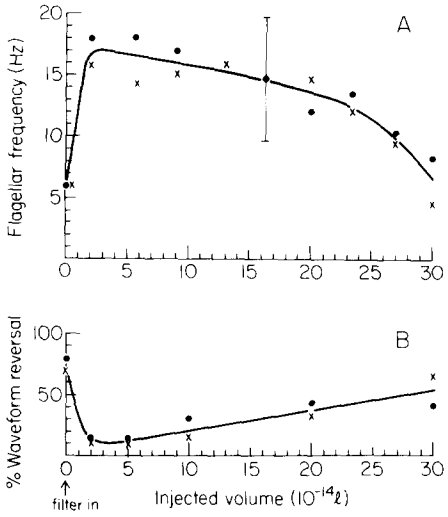

FIGURE 10 Flagellar frequency and percent waveform reversal as a function of the injected volume (average of 21 cells), of either 0.1 M $MgSO_4$ (●) or 0.1 M $MnSO_4$ (×).

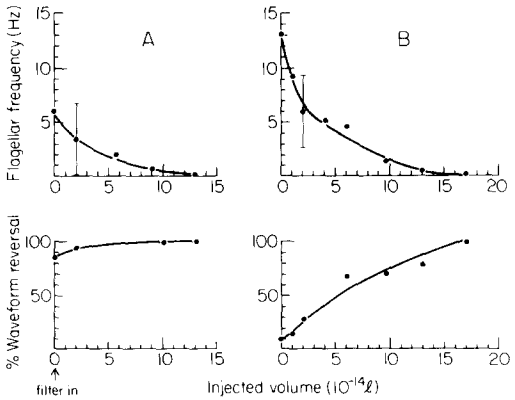

FIGURE 11 Effects of the microinjection of 0.02 M Ca^{2+} on the flagellar activity of impaled *Euglena* under restricted wavelength illumination (*A*) and full illumination (*B*).

mination (420–700 nm), the injection of 4×10^{-14} liter of 0.02 M $CaCl_2$ into *Euglena* caused a 50% decrease in flagellar frequency and an increase in flagellar reversal of from 10% before injection to >40% after injection. Therefore, Ca^{2+} inhibited flagellar frequency and caused waveform reversal under both full and restricted wavelength bands of illumination.

Effects of Various Wavelengths

To investigate the photoinduced response of the *Euglena* flagella as a function of wavelength, it would be desirable to scan the spectrum with a narrow wavelength band. The amount of illuminating light in a narrow (e.g., 20-nm) band is, however, insufficient by a large margin for filming of the flagella.

As an alternative to scanning with a narrow band, we adopted the technique of changing the low wavelength cutoff of the illuminating light with glass filters (Fig. 1). Except for 610–700- and 630–700-nm bands, filming of the flagellar activity was possible in all cases. To record data for the 610–700- and 630–700-nm wavelength bands, the following technique was employed: After the *Euglena* had established a steady state under illumination in either of the wavelength bands, the glass filter (RG610 or RG630) was suddenly (in <20 ms) removed from the light path. The flagellar activity in the first 200 ms after the removal of the filter was taken as representative of the activity during the presence of the filter. Because the response of the flagellum to a change in illumination is delayed at least 1 s, as mentioned in Initial Observations, this appears to be justified.

Fig. 12*A* and *B* shows the flagellar frequency and the percent of cells with waveform reversal of impaled *Euglena* in MM with 1.5 mM EGTA and 10 mM ATP as a function of the low wavelength cutoff. The action spectrum is given by the changes in flagellar activity as a function of the wavelength cutoff, i.e., by the derivative of the lines drawn in Fig. 12*A* and *B*. The data in Fig. 12*A* and *B* indicate that there are two areas in the visible spectrum that affect the flagellar activity. Light in a band centered on ~520 nm causes the flagellar frequency to increase and the waveform to become normal because, in the absence of this light, the flagellar frequency is low, and the waveform is reversed. Analogously, flagellar reversal and a decrease in flagellar frequency are caused by light in a wavelength band centered on ~610 nm because,

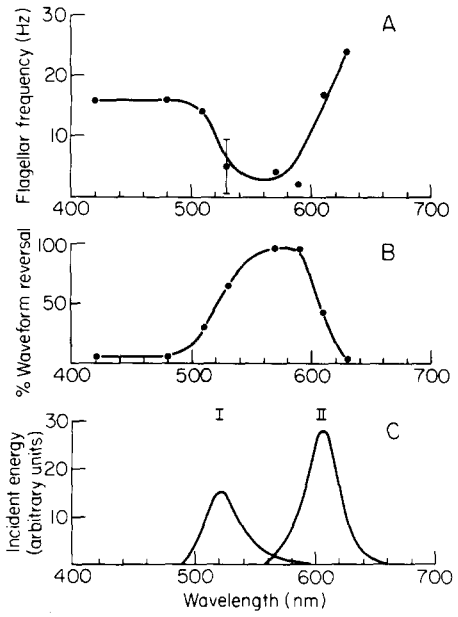

FIGURE 12 Flagellar frequency (*A*) and percent wavelength reversal (*B*) of impaled *Euglena* as a function of the low wavelength cutoff of the illuminating light. The action spectrum is given by the derivatives of the lines drawn in *A* and *B*, as explained in the text. The narrow wavelength bands isolated by combinations of glass and interference filters and used in the experiments described in the text are shown in *C*.

in the absence of this light (when the wavelength cutoff is at 630 nm), the flagellar frequency is high, and the waveform is forward.

To investigate the effects of the light in the above two spectral areas, the narrow wavelength bands shown in Fig. 12*C* were isolated with combinations of glass and interference filters as described in Apparatus. The filter combinations were placed edge to edge in the arrangement described in Fig. 2. This allowed illumination of the preparations with light from either band I or band II, or by a mixture of the two. Under the narrow-band illumination, not enough light was available for filming. By visual observation, however, it could be ascertained whether the flagellar waveform of the *Euglena* was forward or reverse.

When impaled *Euglena* in MM with 1.5 mM EGTA and 10 mM ATP were illuminated with light in band I only, all flagella had forward waveforms. When the illumination was changed to band II, all flagella reversed. By switching the illumination back and forth between bands I and II, the flagellar waveform switched back and forth

between forward and reverse. An apparently indefinite number of these cycles could be produced in a *Euglena*.

When the *Euglena* were illuminated by band I, and therefore exhibited forward waveforms, the admittance of ~60% or more of the light from band II caused the flagellar waveforms to reverse. When the *Euglena* waveforms were reversed by illumination in band II, the change to forward waveforms occurred when ~30% of the light from band I was admitted. Since the percent of mixed illumination was judged by eye, these results should be considered to be qualitative. The results are in agreement, though, with the observation that, under full illumination (420–700 nm), when the intensities of light in bands I and II reaching the preparation are approximately equal, the flagellar waveform is forward.

DISCUSSION

The experiments summarized in Fig. 8 show that, when the external Mg^{2+} concentration in the medium for impaled *Euglena* is 4 mM or greater, the photoinduced effects on the flagellar activity are abolished. As a result of the leaky junction between the impaling microelectrode and the *Euglena* membrane, the inside of the impaled cells is in free diffusional equilibrium with the outside medium (22, 24). The concentration of Mg^{2+} in the interior of the impaled cells is, therefore, similar to the external concentration. The data shown in Fig. 8 thus indicate that a site on which the photoinduced mechanism operates becomes saturated when sufficient Mg^{2+} (>4 mM) is present. The photoinduced flagellar reversal is apparently caused by removal of Mg^{2+} from an internal site or organelle in the *Euglena*. This is supported by the observation (Fig. 10) that the photoinduced flagellar effects can be abolished by the microinjection of Mg^{2+}.

Previously, we have reported experiments (22) in which the flagellar frequency of *Euglena* was lowered by passing electric current through the impaling electrode. It was concluded that the mechanism of this electrical control operated through the removal of Mg^{2+} from an interior site in the *Euglena*. The reduction of flagellar frequency by the two mechanisms, photoinduction and application of electric current, could well operate through a common site. The observation that Mn^{2+} is an apparently complete analogue for Mg^{2+} in abolishing the photoinduced effects, as described above, as well as the recent electric current experiments (22) would support the existence of a common site for both mechanisms.

The medium in which the experiments on impaled *Euglena* were performed contained 1.5 mM EGTA. The concentration of free Ca^{2+} in the medium can be estimated as $\sim 10^{-9}$ M (23). Because there is free exchange between the interior of impaled *Euglena* and the external medium, the concentration of free Ca^{2+} inside the cells should be comparable to the 10^{-9} M concentration in the medium. This concentration is well below the range of 10^{-6}–10^{5} M in which Ca^{2+} is effective in controlling muscle and ciliary activity. It would appear, therefore, that Ca^{2+} is not involved in the Mg^{2+}-mediated mechanisms discussed above.

The microinjection of Ca^{2+} caused a decrease in flagellar frequency independent of the type of illumination prevailing. Flagellar waveform reversal was either initiated (under full illumination, 420–700 nm) or enhanced (under restricted illumination, 530–700 nm) by the microinjection of Ca^{2+}. This makes it probable that the actions of Ca^{2+} are mediated through a different site than the Mg^{2+}-dependent photoinduced effects discussed above, which did not involve Ca^{2+}.

It is possible to speculate on the mode of action of Mg^{2+} in mediating the photoinduced effects on the *Euglena* flagellar activity. *Euglena* possess a paraflagellar swelling that has been shown to contain the primary photoreceptor pigments, flavin chromophores bound to protein macromolecules, responsible for phototaxis in *Euglena* (7). The paraflagellar swelling is located within the membrane of the locomotory, emergent flagellum near its base. Separating the paraflagellar swelling from the flagellar axoneme is the paraflagellar rod, a structure that extends almost the length of the flagellum (3, 33). According to the results presented in the previous section, the increase in curvature of the flagellar waveform in response to a 530–700-nm wavelength band of illumination occurs at the proximal end of the flagellum near the insertion point and the location of the paraflagellar swelling. The Mg^{2+} involved in the control of the waveform reversal response could act at the site of the paraflagellar swelling and rod. The paraflagellar rod has been shown to be attached to the axonemal microtubules (4).

The flagellar activity of *Euglena* cells can be electrically controlled by the application of direct current. It was concluded (22) that this control process operates through a change in the internal concentration of Mg^{2+} in the *Euglena* as a response

to the electrical potential induced by the current. Similarly, the *Euglena* flagellar response to a 530–700 nm-wavelength band of illumination could be the result of a change in potential induced in the photosensitive apparatus (triggered by the restricted illumination). The induced potential would affect the availability of Mg^{2+} to operate in the control of motility.

The action spectrum of the *Euglena* photoprocesses should be related to the absorption spectrum of pigments involved in these processes. The stigma contains β-carotene, lutein, and cryptoxanthin (2), and the paraflagellar swelling has been shown to contain flavins (1, 7, 9). The absorption spectrum for these pigments shows a major peak at 430–500 nm (1, 9, 10, 34) and a minor peak beyond 600 nm (1, 34). This absorption spectrum is compatible with the observation, illustrated in Fig. 12 *B*, that the *Euglena* display forward waveform when illuminating light in the 420–500-nm range is present.

It is unclear why forward flagellar waveforms occur with illuminating light of wavelength longer than 620 nm. It has been suggested that this response may be the result of heat energy, energetic interference between two different pigments, or the presence of other light-absorbing molecules within the cell (1). Checcucci et al. (6) have shown that the red-light-induced phototactic response of *Euglena* is the result of an O_2 effect. Other experiments have indicated that the positive phototactic response in red light was the result of chloroplast contamination (2) or of an auxiliary pigment system located in the eyespot granules (1, 30). This pigment system remains unidentified, however.

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