REVERSIBLE INHIBITION OF *CHLAMYDOMONAS* FLAGELLAR SURFACE MOTILITY

ROBERT A. BLOODGOOD, ELLA M. LEFFLER, and ANNA T. BOJCZUK

From the Department of Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT

Chlamydomonas exhibits force transduction in association with its flagellar surface; this can be visualized by the saltatory movements of attached polystyrene microspheres. This flagellar surface motility has been quantitated by determining the percentage of attached microspheres in motion at the time of observation (60% in the case of control cells at 25°C). A number of experimental treatments reversibly inhibit flagellar surface motility. These include an increase in sodium or potassium chloride concentration, a decrease in temperature, or a decrease in the free calcium concentration in the medium. Many of the conditions that result in inhibition of flagellar surface motility also result in an induction of flagellar resorption. Although both flagellar stability and flagellar surface motility are dependent on the availability of calcium, the two processes are separable; under appropriate conditions, flagellar surface motility can occur at normal levels on flagella that are resorbing.

Inhibition of protein synthesis results in a gradual loss of both the binding of microspheres to the flagellum and the flagellar surface motility. After resumption of protein synthesis, both binding and movement return to control levels. The effect of the inhibition of protein synthesis is interpreted in terms of selective turnover of certain components within the intact flagellum, one or more of these components being necessary for the binding of the microspheres and their subsequent movement. If this turnover is inhibited by keeping the cells below 5°C, the absence of protein synthesis no longer has an effect on microsphere attachment and motility, when measured immediately after warming the cells to 25°C.

KEY WORDS flagella · *Chlamydomonas* · flagellar surface motility · flagellar protein turnover · calcium · polystyrene microspheres

It has recently become apparent that many cases of eukaryotic cell motility occur in close association with biological membranes. Three major classes of such membrane-associated movements can be distinguished: (*a*) the movement or deformation of large areas of plasma membrane, (*b*) the intracellular movement of membrane-bounded organelles, and (*c*) the movement of cell surface receptors for immunoglobulins, lectins, and hormones along the plane of the plasma membrane. This last system is particularly intriguing mechanistically because it is presumed to represent the coupling of intracellular machinery with the movement of cell surface ligands (22). Most cases of receptor motility involve the general cell surface; this creates certain technical difficulties in terms of identifying and isolating the components responsible for the motility. In addition, it has not

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been possible to directly observe the movement of individual receptors.

Bloodgood (3) reported the existence of a motile system involving rapid, local, and bidirectional movements of exogenous marker particles along the external surface of the *Chlamydomonas* flagellum. It was argued that this system involved continuous energy transduction occurring in association with the flagellar membrane and the structures connected with the internal and external surfaces of this membrane. Further, it was argued that the *Chlamydomonas* flagellum can serve as an experimentally manipulatable system for studying rapid, membrane-associated motility.

The present paper represents a quantitative analysis of *Chlamydomonas* flagellar surface motility utilizing a number of conditions that can reversibly inhibit this motile phenomenon.

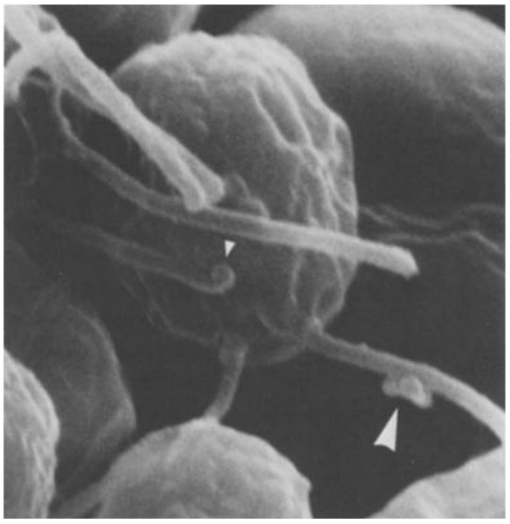

FIGURE 1 Scanning electron micrograph of *Chlamydomonas reinhardtii* strain pf-18 fixed while actively moving 0.35-μm diameter polystyrene microspheres (arrows). × 8,500.

MATERIALS AND METHODS

Cell Strains and Culture Conditions

All data were obtained using vegetatively grown *Chlamydomonas reinhardtii* strain pf-18. This is a paralyzed flagellar mutant in which the flagella are straight. The mutant contains a structural defect in the central structures of the axoneme and is defined as 9 + 0 (26). The central pair of microtubules and the central sheath material are replaced by an amorphous core of material (36). Flagellar surface motility occurs in vegetative and gametic cells of wild type *Chlamydomonas reinhardtii* along with all paralyzed flagellar mutants examined (3).

Chlamydomonas were grown at 22°C in Medium I of Sager and Granick (28) using an alternating cycle of 14 h of light and 10 h of darkness. All experiments were done between the 2nd and 8th h of the light cycle using single cells of uniform size, each possessing two flagella at least 10 μm in length. All cells were washed with fresh growth medium before use, and all experiments were performed in growth medium except where otherwise noted.

Quantitation of Flagellar Surface Motility and of Marker Attachment

Flagellar surface motility was monitored by the bidirectional, saltatory movements of 0.35-μm diameter polystyrene monodisperse microspheres (Polysciences, Inc., Warrington, Pa.) observed using phase-contrast microscopy at a minimum magnification of 600. Provided for purposes of orientation, Fig. 1 is a scanning electron micrograph of *Chlamydomonas reinhardtii* strain pf-18 with polystyrene microspheres associated with the flagellar surface. These cells were exhibiting active flagellar surface motility at the time of fixation. All observations of living cells were made using glass microscope slides that had been cleaned in 7-X detergent (Linbro Scientific, Inc., Hamden, Conn.), extensively washed with distilled water, and stored in 0.5 mM EDTA. This treatment of the slides is necessary to completely eliminate flagellar swelling and detachment during the period of observation. The velocity of polystyrene microsphere movements appears to be invariant and occurs in both inward and outward directions along the flagellum at a velocity of 1.8 ± 0.4 μm/s.

The flagellar surface motility is quantitated by determining the percentage of polystyrene microspheres attached to the flagella that were in motion at the time of observation. Microspheres mechanically coupled to the flagellar surface do not exhibit the Brownian movement characteristic of microspheres free in the medium. The control level of flagellar surface motility for pf-18 cells in growth medium at normal laboratory temperature (23°–25°C) is 59.1 ± 5.2%. Under optimal conditions, any attached microsphere, if observed long enough, will undergo periods of movement. Therefore, the figure of ~60% is taken to mean that attached microspheres spend, on the average, 60% of the time in motion. This figure represents the average of data obtained by three different observers using 40 separate cultures of cells and sample sizes ranging from 100 to 300 attached microspheres scored for each determination. The reproducibility of the experimental data from one batch of cells to another is indicated in Fig. 5, where the three different symbols represent data from three different experiments performed by the same investigator. For each data point in this report, a minimum of 100 microspheres attached to

flagella were scored, requiring an average of 10 min of observational time. Except where otherwise noted, all quantitation of flagellar surface motility was performed at 23°–25°C in culture medium and within 30 min of exposing the cells to the experimental condition. All statistics are expressed in terms of standard deviation.

The quantitation of the attachment of polystyrene microspheres to the flagellar surface is expressed as the number of attached microspheres (both moving and nonmoving) per flagellum. A minimum of 150 cells (300 flagella) were scored for each data point. For the experimental studies, the concentration of cells, the concentration of microspheres, and the volume of medium placed on each slide were all maintained constant.

Flagellar lengths were determined using a Zeiss ocular micrometer that had been previously calibrated with a Zeiss stage micrometer. A minimum of 25 flagella were measured for each data point.

The temperature studies utilized a TS-2 Thermal Microscope Stage (Bailey Instruments Co., Inc., Saddle Brook, N. J.).

Protein Synthesis Measurements

The radioactive labeling technique of Lefebvre et al. (16) was utilized. In preparation for labeling experiments, cells were grown in medium with 90% of the sulfate replaced by chloride. The sulfur-starved cells were incubated at 25° or 2°C in growth medium totally lacking sulfur. At various times after addition of an aliquot of carrier-free [^{35}S]sulfuric acid (New England Nuclear, Boston, Mass.; sp act 43 Ci/mg), duplicate 25-μl samples were removed and spotted on Whatman 3MM filter disks, and the disks placed in ice-cold 10% TCA. The disks were processed by the method of Mans and Novelli (18) and the radioactivity was determined in Liquifluor using a Beckman LS-8100 liquid scintillation counter (Beckman Instruments Inc., Fullerton, Calif.).

Preparation of Cells for Scanning Electron Microscopy

Chlamydomonas reinhardtii strain pf-18 cells were mixed with polystyrene microspheres in growth medium under conditions allowing maximal flagellar surface motility. An equal volume of fixative containing 5% glutaraldehyde and 20 mM potassium phosphate at pH 7.0 was added to the cells. After 30 min of fixation at room temperature, the cells were thoroughly washed in 10 mM phosphate buffer and allowed to settle onto a Millipore HA filter. The cells were fixed on the filter with 1% osmium tetroxide in 10 mM potassium phosphate at pH 7.0. The filter was washed in buffer, dehydrated in Ethanol, transferred to Freon 113, and critical point dried using Freon 13. The filter was then coated with gold in a vacuum evaporator and photographed in a Coates and Welter Cwik Scan scanning electron microscope.

Calculation of Free Calcium Concentrations in EGTA-Calcium Mixtures

The free calcium concentration present in mixtures of calcium and EGTA was calculated using the methods of Portzehl et al. (23). The following approximation was used:

$$[Ca^{2+}]_{free} = \frac{R}{K_{app}(1-R)},$$

where $R = [Ca^{2+}]_{total}/[EGTA]_{total}$ and is kept below 0.9, and K_{app} = apparent dissociation constant for EGTA and calcium and was calculated to equal $10^{6.28}$ at pH 6.8 (pH of the growth medium). The effect of the citrate concentration in the growth medium on the free calcium concentration in the calcium-EGTA mixtures was negligible. Since the ratio of the apparent dissociation constants of EGTA for calcium and magnesium is $\sim 10^5$ (23), the magnesium concentration in the medium (1.2 mM) did not compete significantly with the calcium (added in a total concentration varying from 0.2–1.0 mM) for binding to the EGTA.

RESULTS

With the quantitation technique described in Materials and Methods, experiments were performed to test the effects of various reagents, drugs, and environmental conditions on flagellar surface motility. The results of these studies are summarized in Table I. The effects of all of the treatments

TABLE I

Summary of the Effects of Various Agents on Flagellar Surface Motility and Flagellar Stability

Treatment	Inhibition of surface motility	Induction of flagellar resorption
NaCl, 50 mM	−	+ (16)
NaCl, 150 mM	+	+ (16)
KCl, 150 mM	+	+ (16)
EGTA-calcium buffers (free calcium concn <1 μm)	+	+ (25)
ATP, 5 mM	+	+ (16)
Sodium citrate, 25 mM	+	+ (16)
Cycloheximide, 10 μg/ml	+	−
Chloral hydrate, 10 mM	+	−
Low temperature (0°–5°C)	+	−
Caffeine, 5 mM	+	+ (14)
Isobutyl methylxanthine, 10 μM	+	+*
Amiprophosmethyl, 10 μM	−	+ (24)
Cytochalasin B, 20 μM, 2 h	−	−
Colchicine, 5 mM, 2 h	−	−

* A. Cowan and P. Lefebvre, Yale University, personal communication.

listed in Table I are totally reversible. Detailed results will be presented from experiments dealing with the effects of: (*a*) salt, (*b*) calcium ions, (*c*) temperature, and (*d*) inhibition of protein synthesis.

Effect of Increased Salt Concentration

Increasing the concentration of sodium chloride in the medium results in an inhibition of flagellar surface motility (Fig. 2). The movement of attached microspheres is totally inhibited at 150 mM sodium chloride; this inhibition is totally reversible upon washing the cells into fresh medium. The dose dependence of the sodium chloride inhibition is independent of whether calcium is added to the medium (Fig. 2, filled vs. open circles) and the inhibition of microsphere movement is not reversed by addition of millimolar concentrations of calcium chloride. The results are the same if potassium chloride is substituted for sodium chloride: again, full inhibition occurs at a concentration of 150 mM. Sodium chloride at 150 mM also reversibly inhibits the binding of polystyrene microspheres to the flagellar surface. The concentrations of monovalent cations given above and in Fig. 2 represent the increase over that already found in the growth medium, which has ~1.7 mM sodium and 1.9 mM potassium.

Sodium chloride and potassium chloride induce flagellar shortening (resorption) in *Chlamydomonas* (16). Fig. 3 shows that the kinetics of resorption are essentially the same when either 50 or 150 mM sodium chloride is added to the medium, although the effect of these two salt concentrations on flagellar surface motility is very different. In the presence of 50 mM sodium chloride, microsphere movement occurs at essentially the control level although these flagella are actively being resorbed. Even after periods of time (2 h) when the flagella have shortened to one-fourth of their control length, normal flagellar surface motility is maintained. On the other hand, 150 mM sodium chloride totally inhibits microsphere movement in addition to inducing resorption. Although increased sodium chloride concentrations in the medium can both induce flagellar resorption and inhibit flagellar surface motility, the concentration of salt necessary for maximal effect in each case is very different.

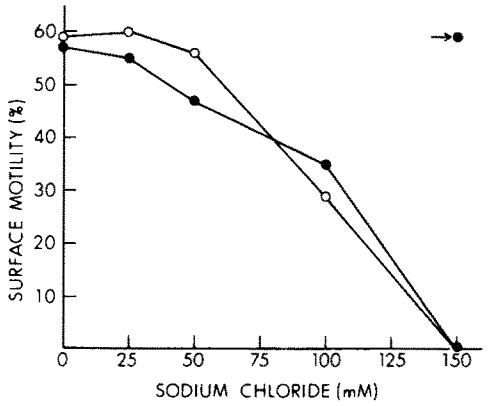

FIGURE 2 The level of flagellar surface motility as a function of sodium chloride concentration. The experiment was performed in the presence (filled circles) and in the absence (open circles) of the normal level of calcium chloride present in the growth medium (0.36 mM). The arrow indicates the level of flagellar surface motility regained after removing cells from medium containing 150 mM sodium chloride.

Effect of Lowering the Free Calcium Concentration

The *Chlamydomonas* growth medium normally contains 0.36 mM total calcium chloride. Excluding the calcium from the medium has little effect on flagellar surface motility. However, if the free calcium concentration of the medium is carefully controlled using calcium-EGTA buffers, a progressive inhibition of microsphere motility is observed as the free calcium concentration is lowered from 10^{-5} to 10^{-6} M (Fig. 4). Below 10^{-6} M free calcium, the level of flagellar surface motility stabilizes at ~30% of its control level (Fig. 4). At all free calcium concentrations tested (EGTA:calcium ratios as high as 11), the inhibition of flagellar surface motility was totally reversed by raising the free calcium concentration of the medium either

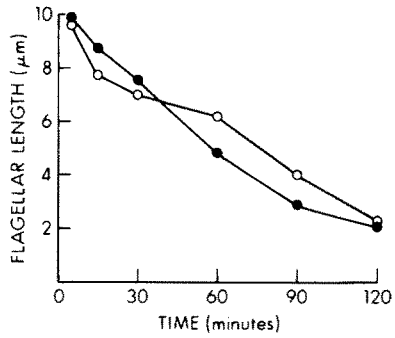

FIGURE 3 Flagellar length vs. time after exposure of cells to 50 mM (open circles) or 150 mM (filled circles) sodium chloride.

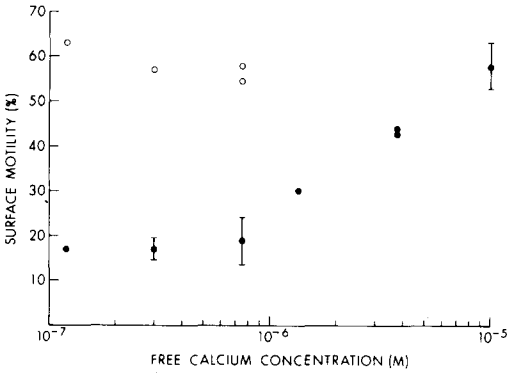

FIGURE 4 The level of flagellar surface motility as a function of the free calcium concentration in the medium (filled circles). The open circles indicate the level of flagellar surface motility after taking cells from the respective experimental samples and returning the level of free calcium to that found in the normal growth medium.

by direct addition of calcium or by washing the cells into fresh growth medium containing the normal level of calcium chloride. The addition to the medium of other chelators of divalent cations such as 5 mM ATP or 25 mM sodium citrate also inhibited the flagellar surface motility. The effects of these agents were reversed by the addition of millimolar calcium. All experiments were performed in the presence of 1.2 mM magnesium.

Effect of Temperature

The level of flagellar surface motility is dependent upon temperature (Fig. 5). Polystyrene microsphere motility is almost totally inhibited at 0°C and the inhibition is rapidly and completely reversed when the temperature is raised. The level of flagellar surface motility appears to plateau above 25°C. The effect of a shift in temperature is seen as soon as the slide is equilibrated to a new temperature using a thermoelectric heating and cooling stage (see Materials and Methods). Cells held for 5 h at a temperature where almost all surface motility is inhibited still regain >90% of the control level of surface motility within 15 min after warming. Changes in temperature have no effect on the level of binding of polystyrene microspheres to the flagellar surface. The observed temperature effects on flagellar surface motility are probably not due to a general depression of cell metabolism and the consequent reduction in available ATP since wild type *Chlamydomonas* can continue swimming at 5°C for several hours.

The temperatures reported in Fig. 5 are the ones indicated by the temperature sensor built into the temperature-controlled stage. Certain independent observations suggest that the temperature registered by the sensor can differ by as much as 5°C from the actual temperature experienced by the cells in the medium between the glass slide and cover glass. When a microscope is equilibrated within a cold room, virtually all flagellar surface motility is observed to be inhibited at a temperature of 5°C, whereas the temperature-controlled stage must register 0°C before inhibition of surface motility is complete. This difference between the temperature registered by the stage and the temperature of the specimen would be greatest at low temperatures and would be expected to be considerably reduced at stage temperatures closer to ambient temperature.

Effect of Inhibition of Protein Synthesis

The effect of cycloheximide on the incorporation of [^{35}S]sulfate into newly synthesized protein in *Chlamydomonas reinhardtii* strain pf-18 is shown in Fig. 6. At 25°C, cycloheximide (10 μg/ml) inhibited 87% of the incorporation of label into new protein. The remaining incorporation of label is attributed to synthesis of protein on chloroplast ribosomes. At 2°C, total cell incorporation of label into protein is inhibited by >98%, both in the presence and in the absence of cycloheximide (Fig. 6).

When *Chlamydomonas* were placed in cycloheximide (10 μg/ml), the flagellar surface motility gradually declined until it was below 5% of the control level after 5 h of incubation (Fig. 7). The effect of cycloheximide on microsphere movement was totally reversed by ~90 min after removal of

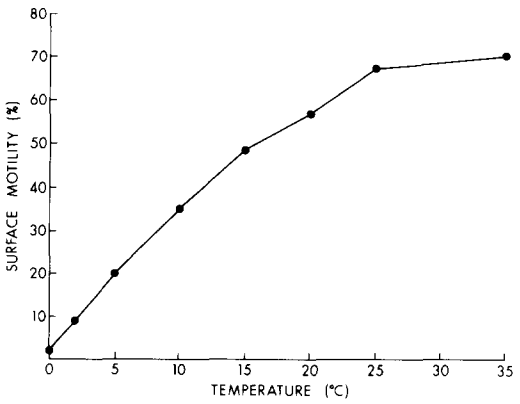

FIGURE 5 The level of flagellar surface motility as a function of temperature.

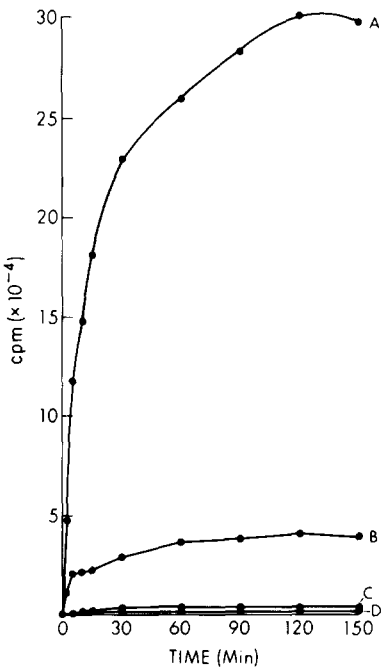

FIGURE 6 Plot of the incorporation of ^{35}S into whole cell protein as a function of time after addition of [^{35}S] sulfuric acid to the medium. *A*, Control cells at 25°C. *B*, Cells in cycloheximide (10 μg/ml) at 25°C. *C*, Control cells at 2°C. *D*, Cells in cycloheximide (10 μg/ml) at 2°C.

the cells from cycloheximide (Fig. 7). No change of flagellar length occurred during 5 h of cycloheximide treatment (filled squares in Fig. 7). In certain experiments, cells were incubated in cycloheximide (10 μg/ml) for 5 h at 2° instead of 25°C, and the cells were then warmed to 25°C and the level of flagellar surface motility immediately measured. It was found that the lower temperature prevented the loss of flagellar surface motility that occurred in the absence of protein synthesis at 25°C (Fig. 8), although protein synthesis was inhibited to an even greater extent (Fig. 6).

It should be noted that the technique used for quantitating flagellar surface motility selects for attached microspheres only and says nothing about the ability of the microspheres to attach to the flagellar surface under the experimental conditions used. During the course of the cycloheximide experiments described above, a drastic decrease in the ability of the microspheres to attach to the flagellar surface was noted. This effect has been quantitated and is linear with time in cycloheximide (Fig. 9). Over a 5-h period, the average number of microspheres attached per flagellum decreased by 75%. This effect is reversible after removal of the cells from cycloheximide (*inset* to Fig. 9). As in the case of flagellar surface motility, incubation of the cells in cycloheximide at 2°C prevented the loss of binding capacity with time in cycloheximide.

The results obtained with cycloheximide were confirmed with another inhibitor of protein synthesis. Chloral hydrate, at a concentration of 10 mM, inhibits protein synthesis in vivo in *Chlamydomonas reinhardtii* without having any effect on RNA synthesis or cell motility (20). This concentration reversibly inhibited flagellar surface motility (Fig. 10) in agreement with the data for cycloheximide. As was the case with cycloheximide, incubation of the cells at 2°C counteracted the inhibitory effect of chloral hydrate on flagellar surface motility.

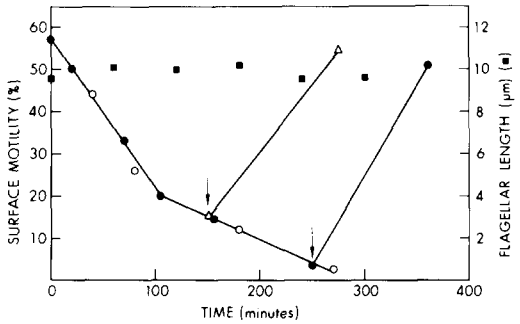

FIGURE 7 The level of flagellar surface motility as a function of time after addition of cycloheximide (10 μg/ml). Open circles, filled circles, and triangles distinguish data points obtained from different experiments. The arrows indicate the times at which samples of cells were removed from cycloheximide. The filled squares indicate the average flagellar length at various times after exposure of the cells to cycloheximide.

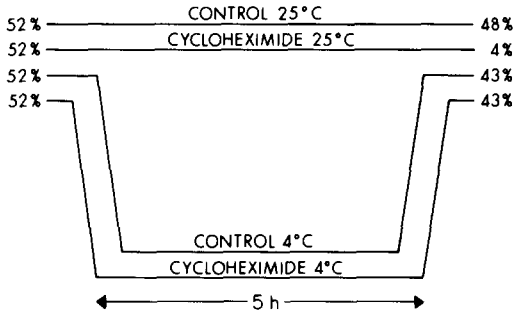

FIGURE 8 Results from an experiment that measured the effects of cycloheximide (10 μg/ml) treatment for 5 h at 25° vs. 2°C on the level of flagellar surface motility.

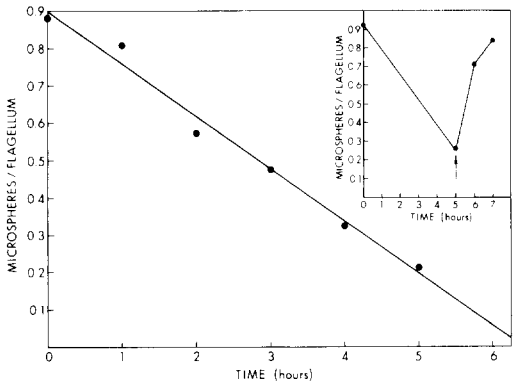

FIGURE 9 The level of adhesion of polystyrene microspheres to the flagellar surface as a function of time after addition of cycloheximide (10 μg/ml). The *inset* shows that this effect is reversed after removal of the cells from cycloheximide (arrow).

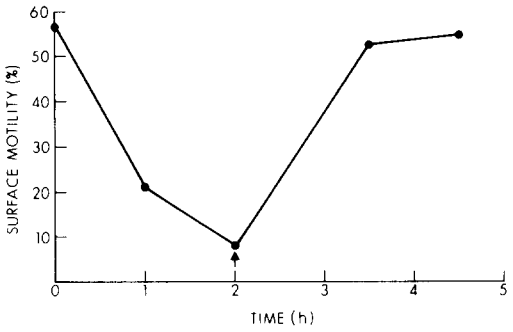

FIGURE 10 Flagellar surface motility as a function of time after addition of 10 mM chloral hydrate. The arrow indicates the time at which the cells were removed from chloral hydrate.

DISCUSSION

Relationship between Flagellar Stability and Flagellar Surface Motility

The effects of EGTA, ATP, and sodium citrate on flagellar surface motility were reversed by raising the free calcium concentration of the medium. It has been shown (16, 25) that these same agents induce flagellar resorption and that this effect is also reversed by addition of calcium. These data suggest that both the stability of the flagellum and its ability to move microspheres along its surface are dependent upon the continued presence of calcium ions.

Although many of the treatments listed in Table I both inhibit flagellar surface motility and induce flagellar resorption, these processes are not obligatorily linked. In the presence of 50 mM sodium chloride, flagellar resorption is maximally induced, but flagellar surface motility is little affected (Figs. 2 and 3). The herbicide Amiprophos-methyl induced flagellar resorption but has no effect on flagellar surface motility during 90 min of exposure, although the flagella have considerably shortened by this time (Table I). Therefore, under appropriate conditions, a flagellum is capable of normal flagellar surface motility while it is undergoing disassembly. Flagellar surface motility also occurs during flagellar assembly (ie., during regeneration after deflagellation) (3).

Are Marker Adhesion and Marker Motility Separable Events?

Under control conditions, virtually every polystyrene microsphere mechanically associated with the flagellar surface undergoes periods of movement. Under appropriate experimental conditions, however, the process of polystyrene microsphere attachment can be uncoupled from the movement of these markers along the flagellar surface. Changes in temperature rapidly alter the level of flagellar surface motility (Fig. 5) without any change in the adhesiveness of the flagellar surface for the polystyrene microspheres. On the other hand, treatment of whole cells with 0.1 mg/ml pronase (a concentration that does not affect the swimming of wild type *Chlamydomonas*) results in a drastic loss of flagellar surface adhesiveness (as measured by the binding of polystyrene microspheres) without any loss in the level of flagellar surface motility (as measured by the percentage of residual attached microspheres in motion). A separation of binding and motility events has also been reported by Hoffman et al. (15), who stated that trypsin inhibits flagellar surface motility without affecting microsphere attachment, while chymotrypsin affects binding of the markers. These same authors claim that colchicine inhibits binding of microspheres while having no effect on the movement of those residual particles that do bind. However, our results indicate that colchicine up to a concentration of 5 mM (far above the concentration necessary to inhibit *Chlamydomonas* flagellar regeneration) has no effect on the binding or the motility of polystyrene microspheres in growth medium over a 2-h period. These experiments were performed with vegetatively grown cells while most of the work of Hoffman et al. (15) dealt with gametic cells.

Protein Turnover in the Intact Flagellum

The most provocative data obtained during this study concern the effect of inhibition of protein synthesis on the flagellar surface motility. Cells suspended in cycloheximide (10 μg/ml) at 25°C maintained fully assembled flagella but these flagella lost both the capacity to bind and the capacity to move polystyrene microspheres (Figs. 7 and 9). These effects were fully reversible after removal of the cells from cycloheximide. Cells incubated in the same concentration of cycloheximide but at 2°C did not lose their ability to bind and to move microspheres, as measured immediately after warming the cells to 25°C (Fig. 8). The results from the cycloheximide experiments were confirmed using an independent inhibitor of protein synthesis in *Chlamydomonas*, chloral hydrate (Fig. 10).

It is hypothesized that there is a constant turnover of certain components within the intact flagellum and that one or more of the components that are rapidly turning over are necessary for microsphere attachment and motility. In the absence of protein synthesis, the turnover results in the exhaustion of any precursor pool of these components within the cell body, after which there is a net loss of these components from the flagellum; concomitant with these events is a loss of microsphere binding and motility. After the removal of cycloheximide and the resumption of protein synthesis, these components are replaced in the flagellum; the flagellum thereby regains the ability to bind and to move microspheres. It is further hypothesized that the turnover of flagellar components is suppressed in the cold and that because of this the absence of protein synthesis has no effect on the microsphere attachment and motility of cells incubated in the cold. These ideas are illustrated in Fig. 11. This diagram suggests that the turnover of components in the intact flagellum results from pinching off of membrane vesicles from the flagellar surface. This process of vesicle release has been reported for both vegetative and gametic *Chlamydomonas* flagella (2, 19, 31). It is, of course, possible that the turnover of flagellar components could be mediated through some endogenous breakdown mechanism. The observations in Figs. 7–10 and the hypothesis in Fig. 11 immediately suggest radioactive labeling experiments designed to identify those components turning over most rapidly within the intact flagellum and hence to identify possible candidates for

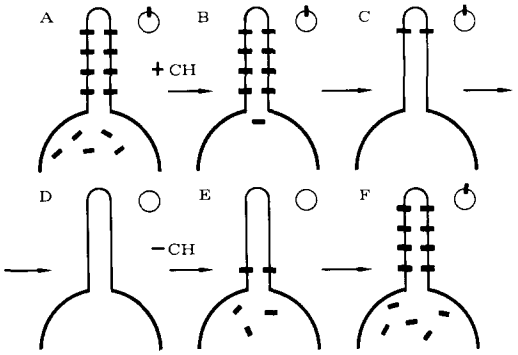

FIGURE 11 Diagram illustrating the manner in which the cycloheximide data from Figs. 7–9 can be explained in terms of protein turnover in the intact flagellum. The solid bars represent a protein component necessary for flagellar surface motility and/or marker adhesion. The small circle next to the flagellar tip represents the budding of membrane vesicles from the flagellar surface. The control situation is presented in Fig. 11 *A*, where the protein component is being inserted into the flagellum from a pool of precursors synthesized within the cell body and then being removed from the flagellum by the pinching off of the membrane vesicles. After addition of cycloheximide (*CH*), protein synthesis is inhibited and the protein component becomes depleted first from the cell body and then from the flagellum (*B-D*). After removal of the cycloheximide and the resumption of protein synthesis, the cellular pool of the component is re-established and subsequently the available sites within the flagellum are filled (*E* and *F*).

proteins involved in the processes of microsphere attachment and movement. Initial pulse-labeling experiments performed in this laboratory using the [^{35}S]sulfate labeling technique (16) have shown (*a*) that label in the form of protein is rapidly incorporated into the intact flagellum, (*b*) that the appearance of these labeled proteins in the flagellum is prevented by cycloheximide, and (*c*) that components of the flagellum are turning over at very different rates. Reports have appeared in the literature suggesting the turnover of protein components in intact *Chlamydomonas* flagella (9, 13) and *Tetrahymena* cilia (21).

Flagellar Surface as a Motor

It has been argued that *Chlamydomonas* flagellar motility involves significant levels of mechanochemical force transduction occurring at or near the flagellar surface (3). Fig. 12 provides a framework for considering the flagellar components that may comprise this motor. The structure labeled *2* in Fig. 12 represents the microtubule-membrane

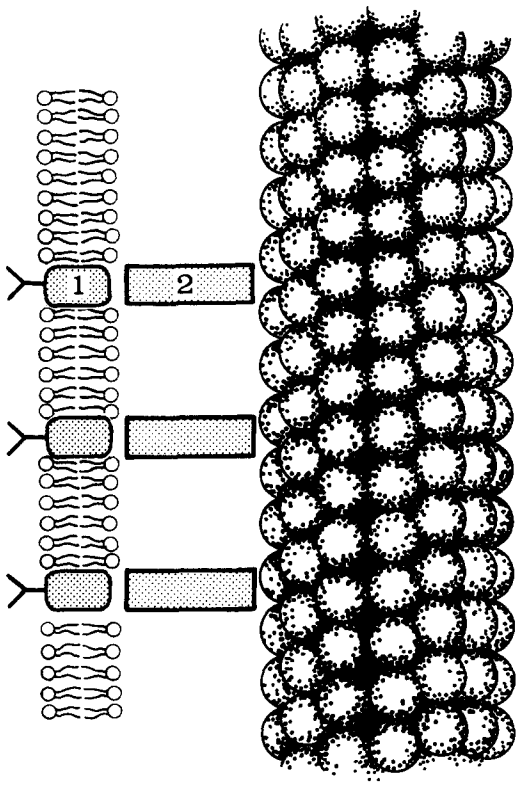

FIGURE 12 Idealized model of the flagellar surface showing the plasma membrane cross-linked to an outer doublet microtubule. Transmembrane glycoproteins (labeled *1*) provide attachment sites for the membrane-microtubule bridges (labeled *2*) and also provide attachment sites (*Y*'s) on the surface of the membrane for attachment of polystyrene microspheres or for attachment of the flagellum to a more physiological substrate.

linkage previously described by electron microscopy to be present in *Chlamydomonas* flagella (27) and *Tetrahymena* cilia (1, 6, 29). This structure may be the site of the calcium-dependent, nonaxonemal ATPase present in the *Chlamydomonas* flagellum (10, 34, 35).

The flagellar membrane of *Chlamydomonas* contains predominantly one polypeptide, which is a glycoprotein migrating on SDS-acrylamide gels with an apparent mol wt >300,000 daltons (2, 31, 36). One reasonable hypothesis is that this large glycoprotein (labeled *1* in Fig. 12) is a transmembrane protein which attaches on the interior surface of the flagellar membrane to an ATPase-containing bridge structure. On the exterior surface of the flagellar membrane, this glycoprotein may provide the binding site for the polystyrene microspheres used in this study as a means of visualizing the flagellar surface motility. This hypothesis carries the experimentally testable prediction that the major membrane glycoprotein is one of the flagellar components that is turning over rapidly and that this protein may be depleted from the intact flagellum during cycloheximide treatment. Treatment of *Chlamydomonas* with Tunicamycin, a specific inhibitor of asparagine-linked glycosylation of proteins, results in an inhibition of flagellar surface motility and flagellar adhesiveness strikingly similar to that which results from cycloheximide treatment (R. A. Bloodgood, unpublished results). These observations suggest that flagellar membrane glycoproteins may be involved in the processes of flagellar surface adhesiveness and surface motility.

Why is There Flagellar Surface Motility?

The observation of a force transducing system at the surface of the *Chlamydomonas* flagellum is unexpected. The question immediately arises as to the function served by this motile system. Two different explanations are consistent with the observations that have been made on flagellar surface motility.

(*a*) Surface motility may be a mechanism for whole cell locomotion. *Chlamydomonas* have been observed to attach to a substrate by their flagellar surfaces and to subsequently glide along this substrate (17; R. A. Bloodgood, manuscript in preparation). A number of the characteristics of this flagella-dependent cell movement (including its velocity and its bidirectionality) suggest that gliding is a physiological expression of the force transduction artificially visualized by using polystyrene microspheres attached to the flagellar surface.

(*b*) Mating in *Chlamydomonas reinhardtii* demands that gametes of opposite mating types become mechanically linked and properly oriented such that their flagellar tips are in register. The initial contact between freely mating gametes seldom occurs such that the flagella interact tip-to-tip. Rather, the flagellum of one gamete is picked up on the flagellar surface motility system of its newly acquired partner and vice versa. The two gametes thus move each other's flagella along their own until a pair of flagellar tips come into contact. At this time, the orientation of that pair of flagella is frozen into position by the action of a gamete-specific locking mechanism (11). This sequence of events has also been observed to occur between mating pf-18 gametes (Dr. G. M. Adams, The

Rockefeller University, personal communication). In fact, it was Lewin (17) who first suggested that the force transduction system involved in *Chlamydomonas* gliding (or creeping, as he called it) was also operative in flagellar alignment during mating.

Relationship between Chlamydomonas Flagellar Surface Motility and Surface Motility Phenomena in Other Cells

Rapid motility of marker objects in association with the extracellular surfaces of membranes is not unique to *Chlamydomonas* flagella, having been observed for the surface of sea urchin blastula cilia (R. A. Bloodgood, manuscript in preparation) and Heliozoan axopodia (4, 33).

In addition, there are at least superficial similarities between the movements of markers along the flagellar surface and the energy-dependent redistribution of cell surface receptors for immunoglobulins and lectins, referred to as capping (for review, see reference 22). In the case of receptor motility on lymphocytes and fibroblasts, it has not been possible to follow the movements of individual receptors, so it is not known whether they perform the rapid, local, bidirectional, and saltatory movements characteristic of the marker particles used with *Chlamydomonas* (and presumably characteristic of the movements of the sites on the flagellar membrane with which the markers associate). In terms of comparing these two force transducing systems, it is instructive to ask whether agents reported in this paper to affect flagellar surface motility have similar effects on capping in mammalian cells. Capping, like flagellar surface motility, is inhibited in the cold (22). In certain cases, cycloheximide has been reported to inhibit capping, but only after long periods of preincubation (8, 22). Removal of extracellular calcium with EGTA has no effect on capping (30, 32), but ionophore-mediated influx of calcium prevents capping and induces dispersion of capped complexes (30). This is in contrast to the observations reported here, where removal of free calcium ions from the medium reversibly inhibits flagellar surface motility. Cytochalasin B (10–20 μg/ml) inhibits capping (7, 22) but has no effect on flagellar surface motility. The mechanism of surface receptor motility in lymphocytes is generally thought to require the active involvement of actin-containing microfilaments but not microtubules (22). Goodenough and Jurivich (12) have reported on a bulk redistribution of antibodies (made to flagellar components) along the flagellar surface and termed this phenomenon "tipping."

CONCLUSION

The *Chlamydomonas* flagellum has many advantages as a system for the study of rapid, membrane-associated motile events. The present study shows that flagellar surface motility can be quantitated, that it requires calcium, and that it is reversibly inhibited by a number of treatments. Most importantly, inhibition of protein synthesis results in the progressive loss from the flagellum of the capacity for polystyrene microsphere adhesion and motility; both of these effects are totally reversible after resumption of protein synthesis. These observations suggest the feasibility of using the incorporation of radioactive labels into the intact flagellum as a means of identifying components that may be associated with flagellar surface motility. The results of the present study argue that the *Chlamydomonas* flagellum is one of the most useful systems in which to study the mechanism of membrane-associated motility.

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