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Source of Energy for Gliding Motility in *Flexibacter polymorphus*: Effects of Metabolic and Respiratory Inhibitors on Gliding Movement

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The effects of selected metabolic and respiratory inhibitors on the gliding motility of *Flexibacter polymorphus* were examined. Motility and oxygen consumption were quantitatively inhibited in a reversible manner by specific respiratory poisons, suggesting that gliding velocity was linked to electron transport activity. Arsenate had little influence on the number or rate of gliding filaments, despite a 95% decrease in the concentration of intracellular adenosine 5'-triphosphate (ATP). At concentrations of cyanide or azide that abolished gliding movement, cells possessed a level of ATP that should have been sufficient to allow motility. Proton-conducting uncouplers of oxidative phosphorylation, such as carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and tetrachlorosalicylanilide, strongly inhibited locomotion yet did not suppress respiratory activity or intracellular ATP sufficiently to account for their effect on movement. Inhibition of motility by CCCP (but not by tetrachlorosalicylanilide) was partially reversed by sulfhydryl compounds. However, unlike CCCP, inhibition of motility by *p*-chloromercuribenzoate, a known sulfhydryl-blocking reagent, was associated with a corresponding reduction in respiratory activity and ATP content of cells. Protein synthesis was not blocked by concentrations of CCCP inhibitory for motility, indicating that utilization of existing ATP in this energy-requiring process was not impaired. These data suggest (but do not unequivocally prove) that ATP may not function as the sole energy donor for the gliding mechanism, but that some additional product of electron transport is required (e.g., the intermediate of oxidative phosphorylation).

Very little is known about the biology of the flexibacteria; even their taxonomy continues to stir controversy from time to time (55). One important diagnostic feature of these procaryotic microorganisms is their readiness to attach to and glide over solid substrata. Elucidation of the mechanics of this gliding process at the subcellular level of organization has proved difficult, primarily because of the lack of flagella or other visible superficial structures that might readily account for movement (4, 6, 10, 19, 21-23, 40, 41, 50; for a review of gliding movement, see Doetsch and Hageage [13]). One aspect of gliding that has received scant attention is the question of the source of metabolic energy driving this process. Cilia and flagella of eucaryotic microbes and the contraction of muscle cells are apparently mediated by a sliding-microfilament mechanism directly activated by adenosine 5'-triphosphate (ATP; 51, 52, 56, 61). Detached cilia and flagella from a number of eucaryotic cell types have been shown to possess adenosine triphosphatase (ATPase) activity and to undergo contraction when ATP is added to suspensions of these motor organelles (20). From respiratory measurements and theoretical considerations, Halfen and Castenholz (24) calculated that in the gliding blue-green alga *Oscillatoria princeps* 0.2 to 5.0% of the total energy generated from oxidative phosphorylation should be sufficient to power its motility. Thus, it is possible that ATP might provide a direct source of energy for gliding movement in such procaryotic microbes. Recent compelling experimental evidence, however, suggests that in *Escherichia coli* and *Salmonella typhimurium* the postulated intermediate form of energy in oxidative phosphorylation (expressed as "~" by Lipmann [37] and Slater [54]), but not ATP, supplies the immediate source of energy for flagella-mediated motility (34). This is in accordance with the apparent lack of ATPase activity associated with in vitro preparations of detached bacterial flagella (1, 12, 14, 45, 63). The energy-linked transhydrogenation of pyri-

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dine nucleotides (11), as well as certain energy-dependent active-transport processes mediated by the cytoplasmic membrane (27, 52), has also been shown to be driven by this intermediate.

Whereas the specific nature of the intermediate is still a matter of conjecture (cf. 28, 39), it might correspond to some non-phosphorylated high-energy compound, a metastable conformational state of the membrane, or an electrochemical gradient imposed across the membrane (for a discussion of these and other possibilities, see the review by Harold [27]). Nevertheless, it is clear that in bacteria the intermediate can be generated either by electron transport or by ATP derived from substrate-level phosphorylation by means of a membrane-bound Mg^{2+},Ca^{2+}-activated ATPase (3). Thus, in the cell, ATP from both glycolysis and oxidative phosphorylation is, normally, reversibly equilibrated with the intermediate.

Herein are reported the effects of selected metabolic and respiratory inhibitors on the gliding motility of *Flexibacter polymorphus*, a recently described filamentous marine species (36). This microbe is particularly well suited for studies on gliding movement since (i) it can be reared in a simple, chemically defined growth medium, (ii) it readily attaches to a variety of solid surfaces, including glass microscope slides, and (iii) it glides swiftly over such surfaces (about 12 μm/s at 23°C), thus allowing accurate rate measurements to be made on many individual filaments in a relatively short time. The data suggest that, as in *E. coli*, the velocity of motility is not correlated with the concentration of intracellular ATP, as measured in the presence of uncouplers of oxidative phosphorylation or inhibitors of electron transport. The possibility that the gliding mechanism is coupled to the intermediate of oxidative phosphorylation, and not to ATP, is discussed.

MATERIALS AND METHODS

Organism and growth conditions. The organism used in this study was *F. polymorphus* (ATCC no. 27820), a heterotrophic, gram-negative marine bacterium (36). This microbe grows in the form of long multicellular filaments, which readily adsorb to and glide over solid surfaces. Cells were grown aerobically in the following defined medium containing (per liter of distilled water): NaCl, 27.0 g; $MgSO_4 \cdot 7H_2O$, 7.0 g; KCl, 0.6 g; $CaCl_2 \cdot 2H_2O$, 0.3 g; tris(hydroxymethyl)aminomethane buffer (Sigma Chemical Co., St. Louis, Mo.), 2.0 g; monosodium glutamate (Sigma), 5.0 g; D-glucose (Mallinckrodt Inc., St. Louis, Mo.), 1.0 g; sodium glycerophosphate (Matheson Scientific, Inc., Los Angeles, Calif.), 0.2 g; vitamin B_{12} (cobalamin), 1.0 μg; trace mineral solution (48), 1.0 ml. The pH of the medium was adjusted to 7.8 before autoclaving. Glucose was not required for growth but was added since it prevented clumping of cells in older liquid cultures. Since motility was poor in older cultures, cells from middle or late exponential phase were used for all measurements. About 0.2 ml of an overnight culture was inoculated into 50 ml of medium in a 125-ml flask and incubated at 23°C on a reciprocating shaker (about 120 oscillations per min). After 18 to 20 h, the optical density of the cell suspension was 0.08 to 0.15 (measured at 580 nm in a Bausch & Lomb Spectronic 20). Cells were used directly from the flask or harvested by centrifugation (5,000 $\times$ *g* for 5 min at 23°C) and resuspended in fresh medium immediately before use.

Motility assay. To assay motility, 0.4 ml of cell suspension (optical density at 580 nm, ca. 0.1) was spread uniformly over the surface of a clean glass microscope slide of standard dimensions (25 by 75 mm), producing a film approximately 0.5 mm thick. Unless stated otherwise, a cover slip was not used, thus allowing rapid diffusion of molecular oxygen to cells and insuring that, from one experiment to the next, preparations were exposed to equivalent oxygen tensions. One or two minutes was allowed for filaments to settle out of suspension and establish contact with the slide surface, since only attached cells exhibited gliding movement; those suspended in liquid on all sides and not touching other filaments or surfaces were nonmotile. The microscope was focused in the plane of the attached trichomes, and the fraction of gliding filaments was determined in a number of randomly selected fields of view (usually about 20). At least 200 attached trichomes were examined for each determination. Rates of gliding of individual filaments were measured with the aid of a calibrated eyepiece micrometer and stopwatch. The number of seconds elapsed to travel 40.2 μm was recorded. A minimum of 25, but usually 40 to 50, observations were made for each determination. All observations were made at room temperature (23 to 25°C) at a magnification of $\times$800 with a Wild microscope equipped with phase-contrast optics. A blue filter and a 2-cm-thick solution of 5.0% (wt/vol) $CuSO_4 \cdot 5H_2O$ were placed between the light source and condenser lens to minimize infrared heating of the microscope stage.

Measurement of respiratory activity. Cells from the late exponential phase of growth were resuspended in fresh medium to an optical density of 0.25 to 0.5 at 580 nm. Oxygen consumption was measured at 23°C in a Clark oxygen electrode (Rank Brothers, Bottisham, Cambridge, United Kingdom) with a strip-chart recorder set at a sensitivity of 10 mV. The electrode had previously been calibrated by a Winkler oxygen titration, using sodium dithionite to establish an anaerobic base line. After obtaining a linear control rate, the respiratory poison or other inhibitor to be tested was introduced into the electrode chamber, containing 2 to 5 ml of cell suspension, and oxygen uptake was monitored for 5 to 10 min. Respiratory rates were reported as percentages of the untreated control rate.

Determination of intracellular ATP. ATP was extracted by injecting 0.1 ml of cell suspension into 5.0 ml of boiling tris(hydroxymethyl)aminomethane buffer (0.02 M, pH 7.75), as described by Hamilton

and Holm-Hansen (25). Duplicate samples were boiled for 4.5 min, transferred to a water bath at 23°C, and immediately assayed for ATP or frozen at −20°C until analysis at a later time. The ATP in the extracts was determined quantitatively, by the luciferin-luciferase assay procedure of Holm-Hansen and Booth (31), using a JRB model 2000 ATP photometer. ATP levels were expressed as percentages of the untreated control value. Internal standards indicated no significant quenching or stimulation of light emission by constituents present in the medium or by the concentrations of metabolic and respiratory inhibitors used in this study.

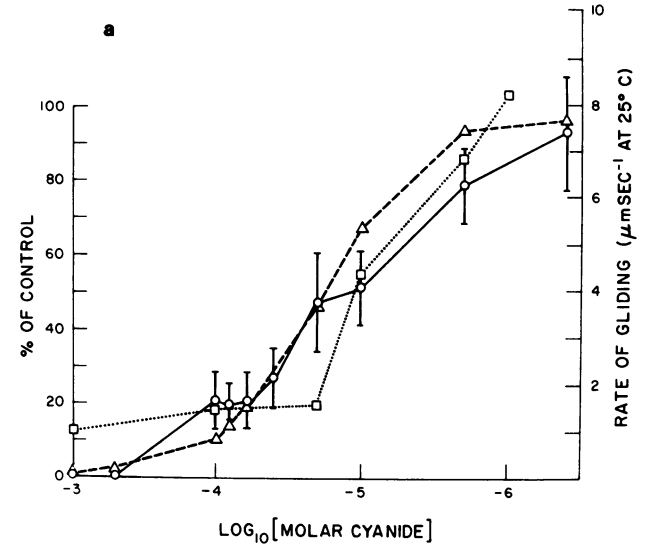
Protein synthesis. Protein synthesis was estimated from incorporation of radioactive lysine into the trichloroacetic acid-insoluble cell fraction. Late-exponential-phase cells were resuspended in 50 ml of fresh medium in a 125-ml flask. With constant stirring, [^{3}H]lysine (0.1 ml of a 0.5-mCi/ml solution; Schwarz/Mann, Orangeburg, N.Y.) was introduced into the cell suspension at $t = 0$, the final concentration of lysine in the medium being approximately 2×10^{-8} M. Portions of 1.0 ml (three for each experimental point) were removed at intervals and mixed with an equal volume of ice-cold 20% (wt/vol) trichloroacetic acid. After 30 min, the precipitate was collected on a 2.4-cm glass-fiber filter and washed with 10 ml of 10% (wt/vol) trichloroacetic acid followed by 10 ml of absolute ethanol. Washed filter pads were placed in scintillation vials with 15 ml of Aquasol (New England Nuclear, Boston, Mass.), and radioactivity was determined in a Beckman model LS-230 liquid scintillation system.

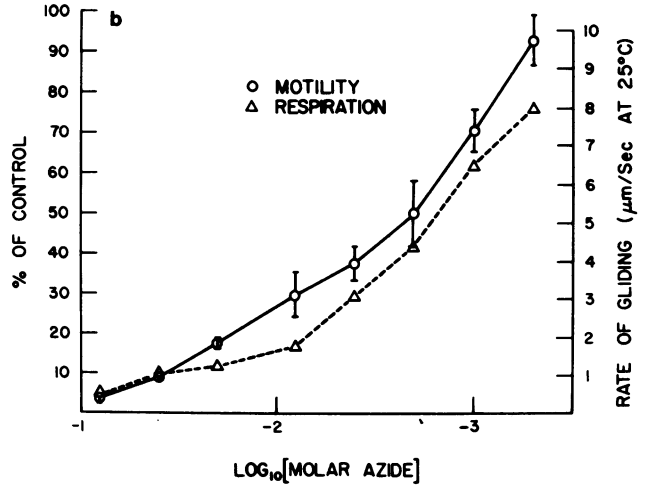
Source and use of inhibitors. Inhibitors were obtained from the following sources: sodium arsenate (Merck & Co., Inc., Rahway, N.J.); sodium azide (Fisher Scientific Co., Fair Lawn, N.J.); sodium cyanide (Allied Chemical Corp., Morristown, N.J.); chloramphenicol (Calbiochem, La Jolla, Calif.); rifamycin (Sigma); mitomycin C (Calbiochem); *p*-chloromercuribenzoate (PCMB; Sigma); carbonylcyanide *m*-chlorophenylhydrazone (CCCP; Calbiochem); carbonylcyanide trifluorophenylhydrazone (FCCP; a gift from P. A. G. Fortes, Department of Biology, University of California, San Diego, Calif.); tetrachlorosalisylanilide (TCS; Sigma); 2,4-dinitrophenol (Sigma); 2-*n*-heptyl-4-hydroxyquinoline *N*-oxide (HOQNO; Sigma). Other agents used were dithiothreitol (DTT; Sigma) and L-cysteine (Matheson Scientific). Rifamycin, CCCP, FCCP, TCS, and HOQNO were dissolved at appropriate concentrations in absolute ethanol. Other compounds were prepared in distilled water, the pH of which was adjusted as necessary with 1.0 N NaOH or HCl. When the effect of an inhibitor on motility was to be tested, it was injected directly into 5.0 ml of cell suspension and mixed vigorously, and the cells were examined for motility at intervals. Low concentrations of ethanol (e.g., 1.0%) had no effect on gliding movement.

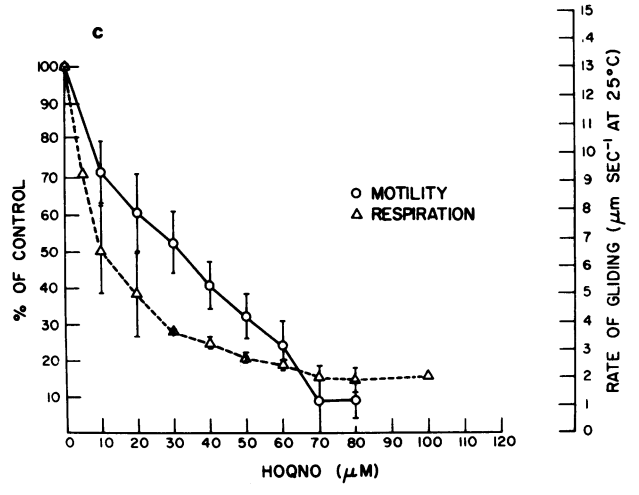
RESULTS

Effect of electron transport inhibitors on motility. *F. polymorphus* is an obligately aerobic microorganism (36). Reduced-minus-oxidized difference spectra of washed, cell-free envelope preparations indicated the presence of a membrane-bound respiratory chain involving *a*-, *b*-, and *c*-type cytochromes (unpublished data). Accordingly, addition of 10^{-3} M sodium cyanide to suspensions of intact cells rapidly blocked uptake of molecular oxygen, presumably by inhibiting a terminal cytochrome oxidase (29). A low level of cyanide-resistant respiration (usually 1 to 3% of the untreated control rate) generally persisted, despite efforts to eliminate it with higher concentrations of inhibitor. Microscopic examination revealed that gliding movement was arrested within 10 s after addition of 10^{-3} M cyanide to the medium. Motility could be fully restored, however, in about 10 min, even after a 2-h incubation, by allowing the cyanide to volatilize from a thin film of cell suspension spread uniformly over the surface of a microscope slide. A large cover glass placed over the cell suspension in such a way as to exclude air bubbles prohibited volatilization of the cyanide, thereby preventing restoration of gliding movement. The rate of gliding movement of individual filaments and the respiratory activity of cells, measured as functions of the concentration of cyanide in the medium, are shown in Fig. 1a. The speed of filament translocation was roughly proportional to respiratory activity (electron transport activity) between 4×10^{-7} and 5×10^{-4} M cyanide. Cyanide concentrations exceeding 5×10^{-4} M abolished all cell movement (including saltatory particle displacement) within a few seconds, whereas concentrations of less than 4×10^{-7} M had no apparent effect on motility. Qualitatively similar results were obtained when cyanide was replaced by sodium azide or HOQNO (Fig. 1b and c). The latter compound, which blocks electron transport in bacteria in the region of cytochrome *b* (29), was effective at

FIG. 1. *(a) Respiratory activity* (△), *ATP pool size* (□), *and gliding rate* (○) *as functions of the concentration of sodium cyanide in the medium. ATP levels represent mean values from two experiments in which ATP was determined 2 to 5 min after addition of cyanide. Motility was measured between 2 and 10 min after adding cyanide to the medium. Oxygen consumption was monitored continuously for 10 min after addition of cyanide. To retard volatilization of cyanide during the motility assay, a cover slip was placed over the cell suspension of the slide. (b) Respiratory activity* (△) *and gliding rate* (○) *as functions of the concentration of sodium azide in the medium. A cover slip was not used in this experiment. (c) Respiratory activity* (△) *and gliding rate* (○) *as functions of the concentration of HOQNO in the medium. A cover slip was not used in this experiment.*


a
% OF CONTROL
RATE OF GLIDING (μmSEC⁻¹ AT 25° C)
LOG10[MOLAR CYANIDE]


b
MOTILITY
RESPIRATION
% OF CONTROL
RATE OF GLIDING (μm/Sec AT 25°C)
LOG10[MOLAR AZIDE]


c
MOTILITY
RESPIRATION
% OF CONTROL
RATE OF GLIDING (μm SEC⁻¹ AT 25°C)
HOQNO (μM)

lower concentrations than cyanide or azide. Motility was also inhibited by anoxia or by perfusion of the medium with nitrogen or carbon monoxide gas, though no attempt was made to quantitate these effects.

The concentration of intracellular ATP rapidly decreased in response to cyanide and other respiratory poisons, presumably reflecting an abrupt shutdown of electron transport activity (Fig. 2). The concentration of ATP was roughly proportional to respiratory activity below cyanide concentrations of 10^{-4} M (Fig. 1a). At higher concentrations of inhibitor, there was little further depletion of ATP, which generally remained around 20% of the initial untreated control value, although, occasionally, values of 25 to 30% were measured, even in 10^{-3} M cyanide. The level of ATP in the presence of 8×10^{-2} M sodium azide (which inhibited the rate of gliding movement by about 96%) likewise fluctuated around 20% of the untreated control value (Fig. 2). Whereas the specific origin of this ATP has not been determined, it could result from a low level of substrate phosphorylation or cyanide-resistant respiration.

The above data strongly suggest that motility is driven by energy derived from oxidation of substrates via an electron transport chain. However, it is also clear from experiments described in a subsequent section (cf. arsenate data) that, even in the presence of respiratory poisons, there should have been sufficient ATP to support gliding movement at the normal (control) velocity. Thus, some additional product generated by electron transport may be required for gliding motility.

Effect of uncouplers of oxidative phosphorylation on motility. Micromolar concentrations of CCCP, FCCP, and TCS uncouple respiratory-chain phosphorylation in intact bacteria and inhibit certain energy-linked active-transport processes, presumably by directly dissipating the energized intermediate of oxidative phosphorylation (27, 28). Each of these compounds strongly inhibited gliding movement in *F. polymorphus*. The concentration dependence of motility inhibition by CCCP is shown in Fig. 3. The rate of gliding decreased to less than 8% of that of an untreated control preparation within 1 min after adding 5×10^{-6} M CCCP to the medium. At a concentration of 10^{-5} M CCCP, the majority of the filaments were immobilized, but those that remained motile glided only at about 2% of the control rate. Low inhibitor concentrations (less than about 4×10^{-6} M CCCP) were associated with uncoupling of oxidative phosphorylation, as judged by a 50 to 60% stimulation in the rate of respiration and a decrease in intracellular ATP (Fig. 3). At

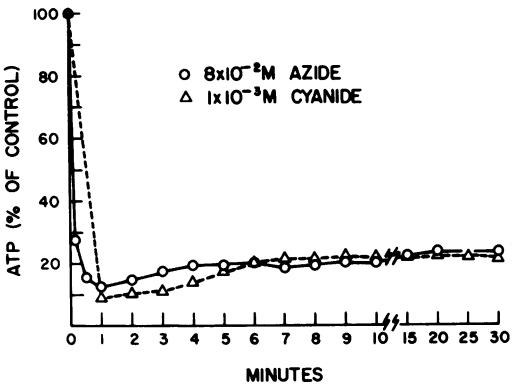


FIG. 2. *Reduction of intracellular ATP in response to 10^{-3} M sodium cyanide* (△) *or 8×10^{-2} M sodium azide* (○) *as a function of time. Inhibitors were added at $t = 0$.*

CCCP concentrations above 4×10^{-6} M, there was a transient stimulation of respiration followed by partial inhibition, suggesting that this uncoupler might interact with the respiratory carriers (Fig. 4). Despite such inhibition, respiratory activity and intracellular ATP generally did not fall below 40 to 50% of their initial untreated control values, even at 2×10^{-5} M CCCP. Similarly, at a concentration of 5×10^{-6} M TCS, which immediately inhibited gliding movement, respiration and ATP remained at about 40% of their control values. Thus, whereas motility was quickly arrested by these uncouplers, sufficient respiratory activity and ATP were present to allow gliding movement, theoretically at about one-half the control velocity (cf. previous section dealing with electron transport inhibitors).

Evidently, CCCP, FCCP, and TCS inhibit motility at a site other than the respiratory chain, possibly by interfering with the coupling and transfer of electron transport energy to the gliding mechanism. Results obtained with 2,4-dinitrophenol were in qualitative agreement with those for CCCP and TCS, except that only partial inhibition of motility was observed.

Sulfhydryl groups, possibly associated with membrane proteins, were apparently involved in the inhibition of respiration and motility by CCCP. Inhibition of these processes by 10^{-5} M CCCP (or FCCP) was partially relieved by the addition of 10^{-3} M DTT (Table 1, Fig. 5). (This concentration of DTT had no effect on inhibition of motility by 5×10^{-6} M TCS.) Interestingly, a recovery in respiratory activity to about 80% of the original untreated control rate led to only about a 27% restoration in the rate of gliding movement. L-Cysteine (10^{-3} M) was about as effective as DTT in this regard, although reversal of inhibition was accompanied

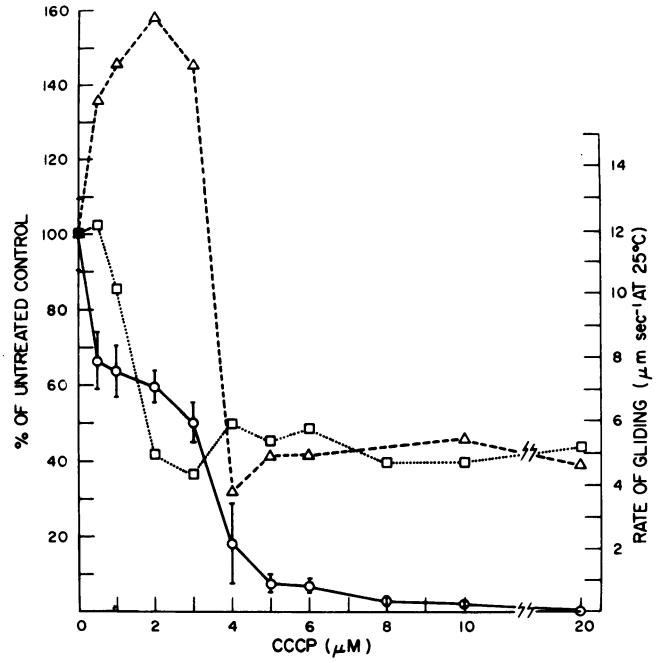


FIG. 3. *Respiratory activity (△), intracellular ATP (□), and gliding rate (○) as functions of the concentration of CCCP in the medium. ATP was determined 10 min after adding CCCP, and respiratory activity was determined after 6 to 8 min. Gliding rates were determined between 3 and 15 min. At a concentration of 10^{-5} M CCCP, most filaments were immobilized. These were not included in the rate calculation.*

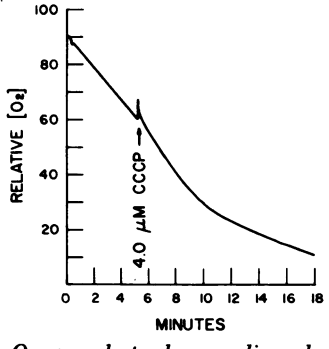


FIG. 4. *Oxygen electrode recording showing initial transient stimulation followed by partial inhibition of respiration by 4 × 10^{-6} M CCCP (cells at an optical density of 0.26 at 580 nm).*

by assimilation and metabolism of this amino acid, complicating interpretation of results. Stimulation of respiration by 2 × 10^{-6} M CCCP, on the other hand, was not suppressed by either of these compounds (Fig. 6). Moreover, when L-cysteine was added, the rate of oxygen uptake increased further, presumably due to metabolism of this substance. These observations suggest that CCCP exerts two distinct effects in *F. polymorphus*, only one of which can be reversed by sulfhydryl compounds.

In view of the above findings, it was of interest to examine the effect on motility of PCMB, a known sulfhydryl-blocking reagent. This compound elicited a time- and concentration-dependent decay not only in the rate of gliding movement, but also in ATP pool size and respiratory activity (Fig. 7). Unlike inhibition by CCCP, inactivation of gliding movement by PCMB could be entirely accounted for by a corresponding loss in respiratory activity of the cells. Incubation of cells in the presence of 10^{-4} M PCMB resulted in a virtually complete cessation of movement and oxygen consumption

TABLE 1. *Reversal of CCCP inhibition of motility by DTT*

Addition	Motility	
	Mean rate (μm/s)[a]	Percentage of control
None (control)	10.6 ± 1.9	100
10^{-5} CCCP	0.26 ± 0.09	2.4
10^{-5} M CCCP; 10^{-3} M DTT after 2 min	2.95 ± 0.57	27.8
10^{-3} M DTT; 10^{-5} M CCCP after 2 min	2.14 ± 0.39	20.2
10^{-3} M DTT	9.59 ± 1.37	90.5

[a] ± Standard deviation.

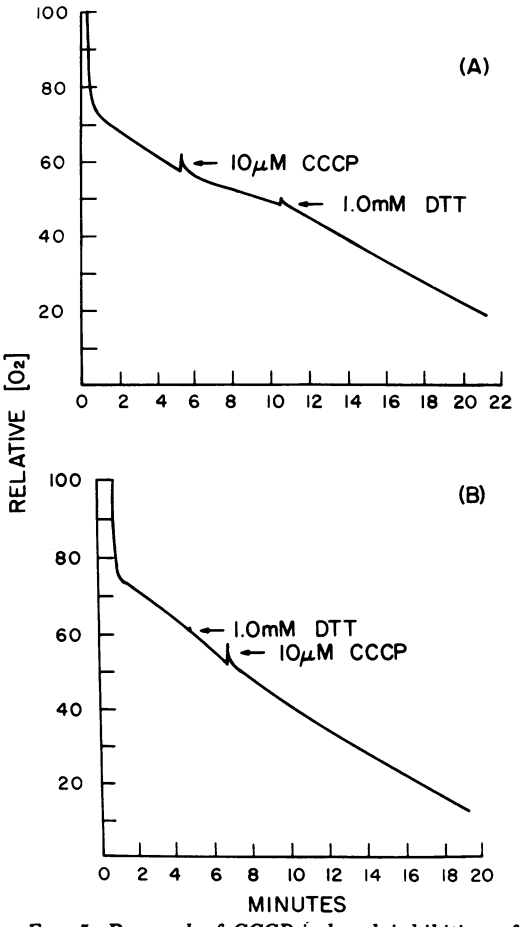


FIG. 5. *Reversal of CCCP-induced inhibition of respiration by DTT. In (A), 10^{-5} M CCCP was added to cells 5 min before adding 10^{-3} M DTT. (B) Reciprocal experiment. (Cells were at an optical density of 0.25 at 580 nm.)*

within about 12 min. During this time, the concentration of intracellular ATP decreased gradually to about 10% of the initial untreated control value, still high enough, theoretically, to permit motility (see arsenate data below). The rates of inactivation of these cellular processes were roughly proportional to the concentration of PCMB in the range of 1×10^{-4} to 3×10^{-4} M and may reflect the kinetics of sulfhydryl binding or the time required for diffusion of a threshold concentration of PCMB to its site(s) of action in the cell. As observed with CCCP or FCCP, it was possible to maintain or partially restore both respiration and motility by adding 10^{-3} M L-cysteine or DTT shortly before or after these processes became inhibited by 10^{-4} M PCMB.

Arsenate reduces the concentration of ATP and phosphoenolpyruvate in intact bacteria (33). Incubation of cells of *F. polymorphus*, which had been reared under phosphate-limiting conditions, in the presence of 10^{-2} M sodium arsenate resulted in a depletion of more than 95% of the intracellular pool of ATP; yet 60 to 80% of the attached trichomes displayed gliding motility at a rate close to 80% of that of the untreated control, even after 2 h (Fig. 8). Respiratory activity of cell suspensions was slightly inhibited in a time-dependent manner by this concentration of arsenate (data not shown), probably accounting for the reduced gliding velocity. Doubling the concentration of arsenate in the medium to 2×10^{-2} M led to a halving of the intracellular concentration of ATP and a similar reduction in the number of gliding filaments, but still did not affect the rate of gliding movement of those filaments that remained motile. Thus, whereas it is possible that some ATP may be required for sustained locomotion,

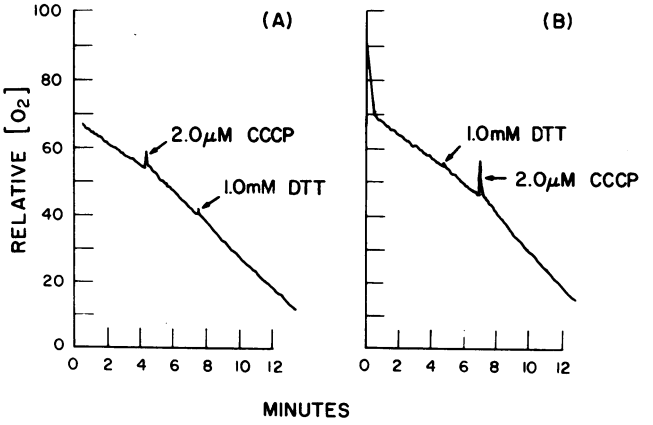


FIG. 6. *Non-reversibility of CCCP-induced stimulation of respiration by DTT. In (A), 2×10^{-6} M CCCP was added to cells 4 min before adding 10^{-3} M DTT. (B) Reciprocal experiment. (Cells were at an optical density of 0.25 at 580 nm.)*

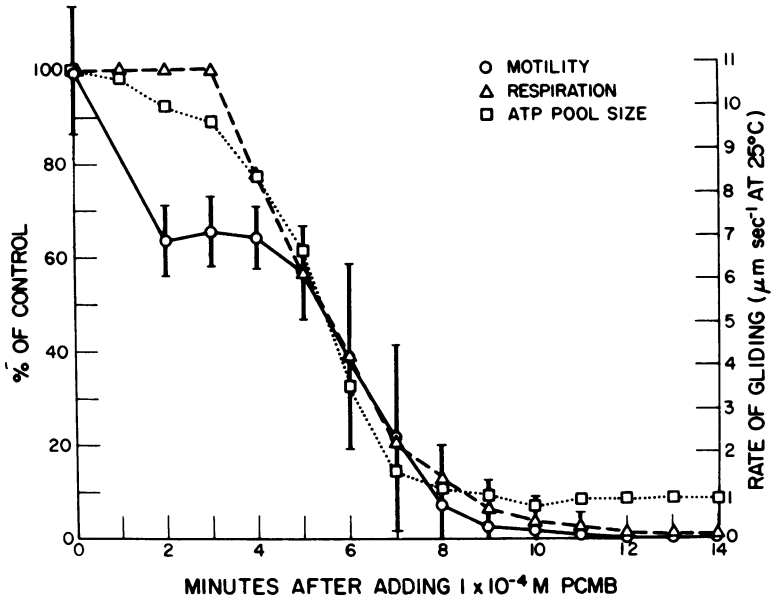


FIG. 7. *Inactivation of respiratory activity* (△) *and gliding motility* (○) *and reduction in the concentration of intracellular ATP* (□) *as functions of the time of incubation in* 10^{-4} *M PCMB. Motility data was averaged from measurements on 24 individual trichomes.*

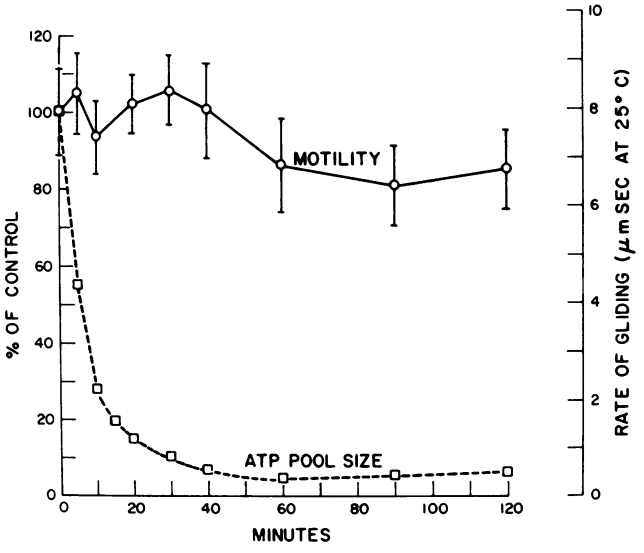


FIG. 8. *Intracellular ATP* (□) *and gliding rate of filaments* (○) *as functions of time of incubation in* 10^{-2} *M sodium arsenate. Approximately 60 to 80% of the filaments were motile at any one time. For this experiment, cells were reared in medium containing 1/20 the normal concentration of sodium glycerophosphate.*

the propulsion mechanism responsible for driving motility apparently is not strongly regulated by the concentration of ATP in the cell. In addition, these data imply that inhibition of gliding movement by respiratory poisons and proton-conducting uncouplers of oxidative phosphorylation, such as CCCP and TCS, cannot be attributed to a reduction in intracellular ATP.

Effect of inhibitors of macromolecular biosynthesis. Chloramphenicol and rifamycin inhibit protein synthesis in bacteria (8, 62). In *F.*

polymorphus, a concentration of 10 μg of chloramphenicol per ml completely blocked incorporation of [^{3}H]lysine into the trichloroacetic acid-insoluble protein fraction within 5 min (data not shown). Apart from a slight reduction in gliding velocity, motility was unimpaired for at least 30 min after the addition of 20 μg of chloramphenicol per ml to cells. The proportion of motile filaments subsequently declined slowly, such that after 2 h approximately 20% of the attached trichomes still exhibited motility at close to the normal (control) rate. Whereas ribonucleic acid synthesis was not measured in the presence of rifamycin, the kinetics of inactivation of gliding movement by this compound (20 μg/ml) was found to be similar to that elicited by chloramphenicol. Thus, protein synthesis does not appear to be directly involved in gliding locomotion.

Effect of CCCP on protein synthesis. Since CCCP inhibited motility without depleting the supply of ATP, it is possible that it interfered with the utilization of ATP by the gliding mechanism. Whereas this possibility was not eliminated directly, the effect of CCCP on protein synthesis was examined under nonrespiring conditions (i.e., in the presence of 10^{-3} M sodium cyanide), since this process requires phosphorylation by ATP during activation and formation of amino-acyl transfer ribonucleic acid complexes (18, 38). The presence of cyanide insured that cells incubated with or without CCCP possessed equal concentrations of ATP (data not shown) and effectively cut off energy produced by electron transport. Cells were permitted to accumulate [^{3}H]lysine in the presence of chloramphenicol (30 μg/ml) for several minutes, washed in unlabeled cyanide-containing medium, and transferred to unlabeled medium supplemented with cyanide and either CCCP (10^{-5} M) or chloramphenicol (50 μg/ml). Transfer of the accumulated radioactive label from the acid-soluble pool into the trichloroacetic acid-precipitable protein fraction was inhibited by chloramphenicol, but largely unaffected by CCCP (Fig. 9). This suggests that CCCP may not inhibit motility by preventing utilization of existing ATP.

DISCUSSION

Since chloramphenicol and rifamycin failed to immediately arrest gliding movement, rapid turnover of cellular protein is probably not necessary for locomotion in *F. polymorphus*. Burchard (5) similarly reported that the gliding movement of *Myxococcus xanthus* was insensitive to chloramphenicol. It is noteworthy that, whereas inhibitors of protein synthesis caused a gradual decline in the number of trichomes that were able to glide, they did not significantly affect the rate of gliding movement. This suggests that these inhibitors did not directly interrupt energy flow to the gliding mechanism. Furthermore, it can be inferred from these results that inhibition of gliding movement by respiratory poisons and uncouplers of oxidative phosphorylation could not have taken place at the level of protein synthesis.

In bacteria, ATP is used in the energization

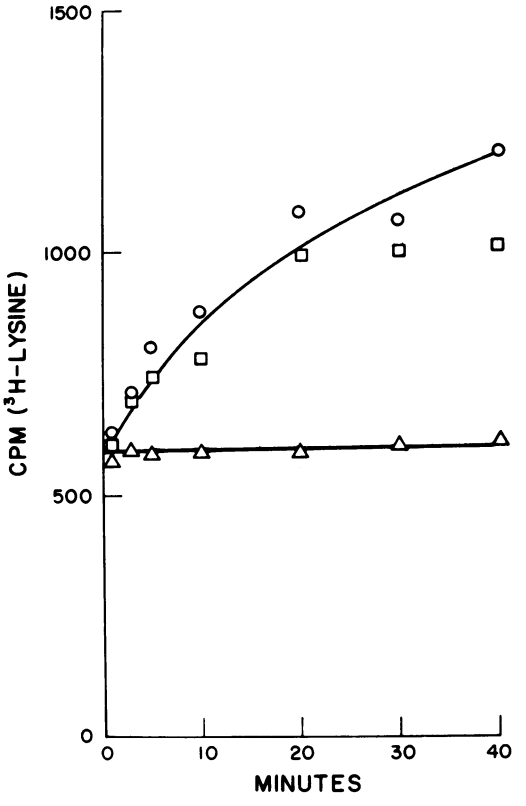


FIG. 9. *Effect of CCCP and cyanide on transfer of accumulated radioactive lysine into the trichloroacetic acid-insoluble cell fraction. Cells were permitted to accumulate [^{3}H]lysine (1.0 μCi/ml, ca. 1.7 × 10^{-8} M) for 9 min in the presence of 30 μg of chloramphenicol per ml (added 5 min before lysine). Sodium cyanide was then added (10^{-3} M), and the suspension was divided into three portions. Exogenous label and chloramphenicol were removed by two washes in unlabeled medium containing 10^{-3} M cyanide. The pellets were suspended in medium supplemented with 10^{-3} M sodium cyanide and either CCCP (10^{-5} M) or chloramphenicol (50 μg/ml). Incorporation of [^{3}H]lysine into the trichloroacetic acid-insoluble material was followed as described in the text. Symbols: 10^{-3} M sodium cyanide only (○); 10^{-3} M sodium cyanide plus 10^{-5} M CCCP (□); 10^{-3} M sodium cyanide plus 50 μg of chloramphenicol per ml (△). (Cells were at an optical density of ca. 0.3 at 580 nm.)*

of the active transport of many amino acids and sugars (2, 33) and in protein and deoxyribonucleic acid synthesis (18). However, for the transport of other amino acids and sugars and for the energy-dependent transhydrogenase reaction, the postulated intermediate of oxidative phosphorylation is utilized directly as the energy donor (53). Like active transport processes, bacterial flagellar motility depends on a continuous supply of metabolic energy to overcome viscous drag forces imposed by the medium (30, 44). Thus, Larsen et al. (34) demonstrated that flagellar rotation in *E. coli* and *S. typhimurium* was linked to the intermediate of oxidative phosphorylation, but not to ATP. Whereas hydrodynamic calculations have indicated that only a very small fraction of the total metabolic energy generated by the cell is required for gliding movement (24), the immediate source of energy for this process has not been previously investigated. Experimental results presented in this paper are consistent with the notion (but do not prove) that gliding motility in *F. polymorphus*, like flagellar motion in *E. coli*, is driven by a product of electron transport (other than ATP) that is dissipated rapidly by specific uncouplers of oxidative phosphorylation. The following lines of evidence tend to support this hypothesis. (i) Respiratory poisons quantitatively inhibited gliding movement and respiratory activity without completely depleting intracellular ATP. (ii) Arsenate (10^{-2} M) reduced the concentration of intracellular ATP below that evoked by respiratory inhibitors or uncouplers of oxidative phosphorylation, yet did not greatly affect the number or rate of motile filaments. (iii) Motility was arrested by low concentrations of proton-conducting uncouplers of oxidative phosphorylation, though, theoretically, there should have been sufficient respiratory activity and ATP available for gliding movement. (iv) At concentrations that inhibited gliding movement, CCCP did not block the utilization of existing ATP in protein synthesis. (Neither CCCP nor TCS inhibits protein synthesis or ATP-dependent uptake of glycerol in *E. coli* [47].) (v) Unlike arsenate (which seemed to elicit an "all-or-nothing" response with regard to motility), the velocity of gliding movement could be regulated by the concentration of CCCP in the medium, independent of intracellular ATP or respiratory activity. This is consistent with the supposition that CCCP dissipates the normal flow of electron transport energy to the propulsion system. The effect of CCCP is similar to that of cyanide and other respiratory poisons that cut off the source of energy to motility. The effect of arsenate could be explained if relatively high concentrations of this compound (e.g., in excess of 10^{-2} M) have a detrimental effect on one or more components of the motility apparatus.

The evidence outlined above is, at best, circumstantial and should be interpreted with some caution since certain important physiological parameters, such as ATP turnover rates (rather than merely pool sizes) and P/O ratios (instead of only oxygen consumption), were not measured. Conceivably, a small, rapidly turning over pool of ATP could generate more energy for gliding motility than a larger, more stable pool. Furthermore, it has not been ruled out that CCCP and TCS could inhibit motility by a mechanism unrelated to energy metabolism, perhaps by causing more general membrane damage leading to partial inhibition of respiration. Moreover, whereas the lack of inhibition by arsenate suggests that phosphorylated high-energy intermediates are not involved in gliding motility, it does not prove this, since the amount of ATP in arsenate-treated bacteria may have provided enough energy for the observed gliding motility. Indeed, both ATP and an energized membrane state could be required for gliding movement. Additional, more refined experiments will be required to test these possibilities. Nevertheless, the limited data at hand can be reconciled with current concepts of energy transformations in biological systems. According to the chemiosmotic hypothesis (42, 43), the intermediate of oxidative phosphorylation corresponds to an electrochemical gradient imposed across the cytoplasmic membrane by the stoichiometric extrusion of protons from the cell, either as a result of movement of electrons down the respiratory chain or by the action of a membrane-bound Mg^{2+},Ca^{2+}-activated ATPase. (*F. polymorphus* possesses a membrane-bound Mg^{2+}, Ca^{2+}-activated ATPase that is insensitive to sodium and potassium ions [unpublished data]. Whereas cyanide [10^{-3} M] partially inhibited this enzyme in vitro, azide, HOQNO, and CCCP had little or no effect on enzyme activity. Thus, inhibition of motility by these agents was probably not directly related to this enzyme.) Uncouplers of oxidative phosphorylation like CCCP and TCS function as lipid-soluble proton ionophores which facilitate electrogenic translocation of protons across the plasma membrane, thereby collapsing the membrane potential (27). Motility in flagellated bacteria is acutely sensitive to low concentrations of such uncouplers (34, 46). A variety of other membrane-active antibiotics and drugs that impede impulse transmission or that depolarize membranes have similarly been shown to inhibit bacterial motility (7, 15, 16). Inhibition of mo-

tility in *Pseudomonas fluorescens* by specific cation ionophores was associated with potassium and proton fluxes across the cytoplasmic membrane (17). Since CCCP and TCS readily inhibit motility in *F. polymorphus*, a transmembrane electrochemical gradient may energize gliding locomotion.

Kaback et al. (32) showed that inhibition of active transport of certain amino acids and sugars by CCCP could be reversed by specific thiol compounds, suggesting that CCCP acts by blocking sulfhydryl groups in proteins important in the transport mechanism per se or in the transformation and coupling of energy to that process. Hancock and Braun (26) likewise demonstrated that inhibition by CCCP of the energy-dependent adsorption of bacteriophages T_1 and $\phi 80$ was reversible by DTT. Since inhibition of motility in *F. polymorphus* by CCCP (or FCCP) was partially reversed by DTT or L-cysteine, it is uncertain whether these agents were blocking sulfhydryl groups involved in the motility mechanism itself or in energy transduction. Whereas we have not distinguished between these possibilities, it is clear that the mechanism of action of PCMB, a known sulfhydryl-blocking reagent, was different from that of CCCP. Inhibition by the former, apart from requiring significantly higher concentrations than CCCP, was strongly time dependent and could be fully attributed to inhibition of electron transport activity. Moreover, inhibition of gliding movement by TCS was not reversed by thiol compounds. In addition, there is evidence that DTT may inactivate CCCP and FCCP in vitro (personal communication, G. W. Ordal, Department of Biochemistry, University of Illinois, Urbana). In view of these data, inhibition of gliding movement by CCCP probably does not reflect a direct sulfhydryl effect on components of the motility system.

The observation that motility and respiratory inhibition, but not respiratory stimulation, were reversed by sulfhydryl compounds suggests that CCCP may simultaneously exert two separate effects in *F. polymorphus*: (i) an uncoupling effect manifested at low concentrations, which leads to respiratory stimulation and motility inhibition and which is insensitive to sulfhydryl compounds, and (ii) a reversible interaction at high concentrations (e.g., above 4×10^{-6} M) with respiratory carriers or an enzyme system at some earlier step (e.g., a dehydrogenase; for an alternative explanation, see Cavari et al. [9]). At high concentrations of CCCP, DTT could partially relieve inhibition of electron transport, thereby increasing the rate of supply of the energized product responsible for driving motility. This is in qualitative agreement with the disproportionate recovery in respiratory activity (elicited by adding DTT to CCCP-inhibited cells), which was associated with a less marked restoration in the rate of gliding movement.

Forcible extrusion of extracellular mucilage from the cell wall has been proposed as a means of propulsion for gliding locomotion in bacteria and blue-green algae (13, 63). In the gliding bacteria that have been examined, such extracellular mucilage is composed of fibers of complex heteropolysaccharide (58, 60). A similar chemical composition appears likely for the extracellular polymeric fibers extruded by cells of *F. polymorphus* (49, 50). A small amount of extracellular sheath material may indeed be required to mediate attachment of the trichome to the glass surface or to facilitate movement of the filament over the slide, and the ability of a cell to glide would thus depend on maintaining some minimum concentration of ATP. However, the present findings argue against movement by forcible sheath extrusion, which would require continuous biosynthesis of polysaccharide at a rate commensurate with gliding velocity. This would imply a positive correlation between the rate of gliding movement and the concentration of ATP in the cell, since the manufacture of exopolysaccharides in general (35, 57), as well as other kinds of extracellular polymeric substances (59), depends on the availability of ATP. Since the rate of gliding movement in arsenate medium was not appreciably affected by a relatively large reduction in the level of intracellular ATP, active mucilage extrusion as a means of propulsion for gliding movement seems improbable.

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