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Relationship Between Proton Motive Force and Motility in *Spirochaeta aurantia*

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The effects of various metabolic inhibitors on the motility of *Spirochaeta aurantia* were investigated. After 15 min in sodium arsenate buffer, 90% of cells remained motile even though adenosine triphosphate levels dropped from 5.6 to 0.1 nmol/mg (dry weight) of cells. After 70 min in sodium arsenate, 5% of cells were motile. Addition of phenazine methosulfate plus ascorbate at this time resulted in motility of 95% of cells, but adenosine triphosphate levels remained at 0.1 nmol/mg of cell dry weight. Carbonyl cyanide-*m*-chlorophenyl hydrazone rapidly (within 1 min) and completely inhibited motility of metabolizing cells in potassium phosphate buffer. However, after 15 min in the presence of carbonyl cyanide *m*-chlorophenyl hydrazone the cellular adenosine triphosphate level was 3.4 nmol/mg (dry weight) of cells, and the rate of oxygen uptake was 44% of the rate measured in the absence of carbonyl cyanide *m*-chlorophenyl hydrazone. Cells remained motile under conditions where either the electrical potential or the pH gradient across the membrane of *S. aurantia* was dissipated. However, if both gradients were simultaneously dissipated, motility was rapidly inhibited. This study indicates that a proton motive force, in the form of either a transmembrane electrical potential or a transmembrane pH gradient, is required for motility in *S. aurantia*. Adenosine triphosphate does not appear to directly activate the motility system in this spirochete.

Spirochetes are chemoheterotrophic bacteria characterized by a distinctive morphology. Generally, a spirochetal cell is helical in shape and flexuous and possesses a protoplasmic cylinder which comprises the nuclear and cytoplasmic regions, as well as the membrane-peptidoglycan layer. Wrapped around the protoplasmic cylinder are structures called axial fibrils or periplasmic fibrils which vary in number from 2 to more than 100 in different spirochetes (8, 12). One end of each axial fibril is inserted near one pole of the protoplasmic cylinder, whereas the other end is not inserted. Inasmuch as each axial fibril is wrapped around most of the length of the protoplasmic cylinder, axial fibrils inserted near one end of the protoplasmic cylinder overlap in the central region of the cell with axial fibrils inserted near the opposite end (8, 11, 12). Both the axial fibrils and protoplasmic cylinder are enclosed by a membrane called the outer sheath or outer cell envelope (8, 11, 12). Axial fibrils have been demonstrated to play a role in spirochetal motility (10, 41). Furthermore, these structures are similar in fine structure and somewhat similar in chemical composition to bacterial flagella (5, 6, 21, 22, 23, 27, 31, 39). Some investigators have referred to these structures as endoflagella or flagella (22, 25, 53); however, axial fibrils differ significantly from bacterial flagella in that they are entirely endocellular organelles.

Apparently, as a result of their cellular architecture, spirochetes exhibit a type of motility unique among bacteria. The spirochetal cell, which does not possess exoflagella, has translational motility in liquids. Additionally, at least certain spirochetes are able to "creep" or "crawl" on solid surfaces (7, 14). Spirochetes also exhibit translational motility in viscous or gel-like environments which immobilize many flagellated bacteria (4, 17, 28). The motions of spirochetes include rotation about the longitudinal axis, propagation of waves, flexing, looping, lashing, whipping, and vibrating (12).

Little is known about the mechanism(s) of spirochetal motility. It has been suggested that the basis of movements in spirochetes is essentially similar to that in flagellated bacteria (2). Existing evidence indicates that bacterial flagella are rigid helices which rotate by means of a "biological motor" at their point of insertion in the cytoplasmic membrane (1, 3, 49). This flagellar rotation is driven by a proton motive force (15, 30, 32, 35, 36, 48). Berg (2) has suggested that the motile behaviors exhibited by spirochetes can be explained if it is assumed that the axial fibrils rotate within the periplasmic space. Alternatively, it has been suggested that some or all of the spirochetal movements are based on some as yet unidentified contractile system which may be somewhat similar to systems involved in eucaryotic cell movements (24). This is of particular interest in light of the fact that it has been suggested that a spirochete or spirochete-like organism was the procaryotic ancestor of eucaryotic flagella (33, 34). Unlike bacterial flagella, eucaryotic flagella propel cells by propagation of waves. The energy for this wave propagation is apparently derived from ATP directly (46, 47, 51).

The purpose of the investigation reported here was to determine the source(s) of energy for motility of the free-living, facultatively anaerobic spirochete *Spirochaeta aurantia*. Such information is essential in developing an understanding of the mechanisms of spirochetal motility. Furthermore, we felt that this information would be of value in gaining insights regarding the evolution of cellular motility systems.

MATERIALS AND METHODS

Bacterial strain and growth conditions. The organism used was *S. aurantia* strain M1 (9). As previously reported, cells of this spirochete generally had two axial fibrils, each inserted near one end of the protoplasmic cylinder and overlapping the other fibril in a 1-2-1 arrangement (9, 16). *S. aurantia* was grown on glucose-Trypticase (BBL Microbiology Systems)-yeast extract (GTY) medium (16) that had been modified by omitting the potassium phosphate. Cells were grown in stationary tube cultures (5 ml of modified GTY medium per test tube [16 by 150 mm]). Incubation was in an air atmosphere at 30°C, and cells were harvested from cultures in the late-logarithmic phase (final culture density; 5×10^8 cells per ml) by centrifugation ($3{,}000 \times g$) for 15 min (16) at 4°C. The inocula (0.1 ml) for these cultures were from similar 5-ml cultures that had been incubated for 24 h.

Preparation of cell suspensions. Generally, cells harvested as described above were suspended in 10 mM potassium phosphate buffer (pH 7.0 unless otherwise specified) containing 5 mM D-glucose. For some experiments cells were suspended in 10 mM sodium arsenate buffer (pH 7.0) and where indicated, either 5 mM D-glucose or 1 μM phenazine methosulfate (PMS) plus 10 mM sodium ascorbate were added to this buffer. *S. aurantia* was suspended to a density of 10^8 to 2×10^8 cells per ml for motility and ATP measurements, to a density of 10^7 cells per ml for measurements of transmembrane electrical potentials and transmembrane pH gradients, and to a density of 5×10^9 to 10^{10} cells per ml for oxygen uptake measurements.

Motility measurements. Generally, cell suspensions were placed in microscope observation chambers and observed immediately (16). Velocities of individual cells were measured as described elsewhere (16, 17). Average velocity was calculated by the method of Kaiser and Doetsch (28). The percent of motile cells in suspension as well as the behavior of cells was determined by direct observation (16, 17) and confirmed by the dark-field photomicroscopy tracking method (30).

Measurement of intracellular ATP levels. ATP was extracted from *S. aurantia* by adding 0.2 ml of cell suspension to 1.8 ml of boiling Tris buffer (0.02 M, pH 7.75, at 25°C) as described elsewhere (44). After 3 min, samples were transferred to a −25°C freezer and stored (up to 10 days) for later analysis. ATP concentrations in these samples were determined by the luciferin-luciferase assay as described by Roberton and Wolfe (45); however, the volumes of sample and firefly extract used in each assay were 1.0 and 0.5 ml, respectively. A liquid scintillation counter (Beckman model LS-230 equipped with a carbon-14 fixed-window isoset module) was used to measure luminescence of firefly extracts.

Measurements of the rate of oxygen uptake. A Clark oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio) equipped with a strip chart recorder set at a sensitivity of 10 mV was used to determine rates of oxygen uptake by suspensions of *S. aurantia*. A solution of sodium dithionite was used to establish an anaerobic base line. Generally, inhibitors were added directly to cell suspensions in the electrode chamber after a linear rate of oxygen uptake had been established.

Measurements of Δψ. Previously described techniques (32) that employ the cationic, lipophilic carbocyanine dye, 3,3′-dipropyl-2,2′-thiodicarbocyanine iodide [Di-S-C_3(5)] were used to monitor transmembrane electrical potential (Δψ) in *S. aurantia*. Di-S-C_3(5) was added to cell suspensions (final concentration, 0.5 μM), and the fluorescence intensity at 660 nm was followed with a Perkin-Elmer model 203 fluorescence spectrophotometer with a strip chart recorder. Excitation was at 620 nm. Di-S-C_3(5) distributes across biological membranes in response to Δψ, intracellular concentrations increasing as Δψ increases (cell interior negative), and uptake of this dye results in fluorescence quenching. Thus, by constructing calibration curves as described elsewhere (32), Δψ was estimated from measurements of Di-S-C_3(5) fluorescence.

Measurement of ΔpH. Fluorescence of the lipophilic weak base 9-aminoacridine was used to follow the transmembrane pH gradient (ΔpH) in *S. aurantia* as described for other biological systems (40). This dye was added to cell suspensions (final concentration, 0.1 μM), and fluorescence intensity at 400 nm was measured by spectrofluorometry as described above. Excitation was at 365 nm. Uptake of 9-aminoacridine serves as an indication of ΔpH since this weak base distributes across biological membranes in response to a chemical proton gradient. As ΔpH (pH inside of cells higher than outside) is increased, internal concentrations of 9-aminoacridine decrease and vice versa. When this dye enters the cell, fluorescence is quenched. Thus, fluorescence intensity can be used as a measure of ΔpH.

Chemicals. Sodium arsenate, sodium ascorbate, PMS, carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP), valinomycin, and 9-aminoacridine were purchased from Sigma Chemical Co. Di-S-C_3(5) was supplied by A. Waggoner, sodium nigericin was obtained from R. Hamill, and bis(hexfluoroacetonyl) acetone (1799) was supplied by E. Lindley. Valinomycin, nigericin, CCCP, 1799, Di-S-C_3(5), and 9-aminoacridine were dissolved in methanol. In cell suspensions containing these chemicals the methanol concentration did not exceed 0.3%. This concentration of methanol did not affect motility of *S. aurantia* M1. Other chemicals were added to cell suspensions as aqueous solutions.

RESULTS

Motility of *S. aurantia*. It was demonstrated by microscopic examination that cells grown in modified GTY medium were motile throughout the logarithmic phase of growth. Over 90% of the cells retained translational motility for at least 5 h after being suspended in potassium phosphate buffer in the presence or absence of D-glucose. The average velocity of cells translating through potassium phosphate buffer plus D-glucose was 26 μm/s. The behavior of *S. aurantia* cells in this buffer was identical to that described by Greenberg and Canale-Parola (16). The organisms moved in straight lines or nearly straight lines. Occasionally a cell stopped momentarily and flexed, and then resumed translational motility. When translational motility resumed, the direction of movement was usually altered, and the previously anterior cell end sometimes became the posterior end of the cell.

Relationship between intracellular ATP concentration, respiration, and motility in *S. aurantia*. Arsenate is known to inhibit the synthesis of high-energy phosphorylated compounds such as ATP and phosphoenol pyruvate in bacteria (29). In fact, when *S. aurantia* was suspended in sodium arsenate buffer, the intracellular ATP concentration dropped from an initial value of 5.6 to 0.1 nmol/mg (dry weight) of cells within 15 min. At this time 95% of the cells remained motile (Fig. 1), but generally they did not exhibit translational motility. Instead, most cells flexed incessantly. Further incubation in sodium arsenate buffer resulted in a decrease in the percentage of cells that were motile. After 60 min, 95% of the cells were immotile. The addition of PMS and ascorbate as an electron donor for these immotile cells resulted in a restoration of translational motility. The average velocity was 23 μm/s, and addition of PMS and ascorbate did not result in a detectable increase in cellular ATP levels. Motility restored by the electron donor was rapidly abolished by the addition of 1799, a proton ionophore (Fig. 1). Thus, motility in *S. aurantia* occurred in the presence of very little ATP as long as the energy from electron transport was available. Apparently, either ATP was not required for motility, or the low levels of ATP present in cells suspended in sodium arsenate buffer were sufficient to support motility.

To further study the role of ATP in motility, the effects of 1799 and CCCP on cells suspended in phosphate buffer plus D-glucose were investigated (Table 1). These proton ionophores serve to uncouple oxidative phosphorylation (19, 50), but in the presence of an energy source such as D-glucose, *S. aurantia* should be able to generate ATP by substrate level phosphorylation. Motility of *S. aurantia* was rapidly abolished (within 1 min) in the presence of 2 μM CCCP or 10 μM 1799 even though ATP concentrations were much higher than those in motile cells in the presence of sodium arsenate. Thus, ATP alone did not appear to serve as the immediate source of energy for motility in *S. aurantia*.

After 15 min in sodium arsenate buffer plus D-glucose, cells exhibited a flexing behavior rather than translational motility. Not only did this correspond to an inhibition of ATP synthesis, but also respiratory activity (rate of oxygen uptake) was inhibited (Table 1). Thus, as ex-

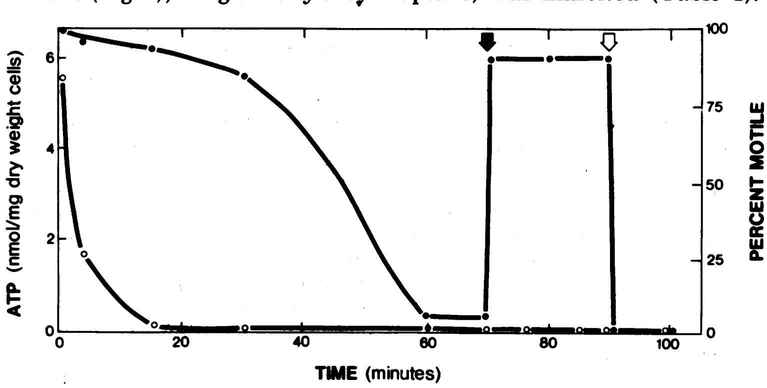

FIG. 1. *Intracellular ATP concentration (○) and percentage of cells that were motile (●) in 10 mM sodium arsenate buffer (pH 7.0). At 70 min (black arrow), PMS and ascorbate were added. At 90 min (white arrow), 1799 was added to a final concentration of 10 μM.*

TABLE 1. *Effects of inhibitors on motility, respiration, and ATP pools in S. aurantia*[a]

Inhibitor	% Motile	Motile behavior	ATP concn (nmol/mg [dry wt] of cells)	Rate of oxygen uptake (%)[b]
None	95	Translating (28)[c]	5.6	100
CCCP (2 μM)	0		3.4	44
1799 (10 μM)[d]	0		4.8	50
1799 (10 μM)	0		1.1	2
Arsenate (10 mM)[e]	95	Flexing	0.1	18
Arsenate (10 mM) plus PMS-ascorbate[f]	90	Translating (23)[c]	0.06	90

[a] Unless otherwise specified, inhibitors were added to cells suspended in 10 mM potassium phosphate buffer (pH 7.0) plus 5 mM D-glucose and motility, ATP, and oxygen uptake measurements were determined after 15 min of incubation with inhibitor.

[b] Measured over a 5-min period. The rate of oxygen uptake in the absence of inhibitors is defined as 100%.

[c] Values in parentheses indicate average velocity measurements in micrometers per second.

[d] Incubation time 1 min rather than 15 min.

[e] Sodium arsenate buffer (10 mM, pH 7.0) was used in place of phosphate buffer.

[f] Sodium arsenate buffer (10 mM, pH 7.0) was used in place of potassium phosphate buffer, and PMS-ascorbate was used in place of D-glucose.

pected, arsenate blocked the metabolism of D-glucose. When the D-glucose in sodium arsenate buffer was replaced by PMS and ascorbate, cells exhibited typical translational motility, and the respiratory activity of these cells approached that of the control cells suspended in potassium phosphate buffer plus D-glucose (Table 1). This suggests that in cells depleted of ATP, respiration is required at least for translational motility. Perhaps the low level of respiration exhibited by cells suspended in arsenate plus D-glucose was sufficient to support uncoordinated flexing. However, respiratory activity alone did not support motility. As expected, CCCP and 1799 did not completely inhibit respiratory activity as they did motility (Table 1). It should be noted that after 15 min in the presence of 1799 the cellular ATP level and rate of oxygen-uptake had decreased (Table 1). Apparently 1799 exerted a secondary effect on *S. aurantia.*

Dark-field photomicrographs (3-s exposure) were used to document the motile behaviors of *S. aurantia* in the presence of various inhibitors (Fig. 2). Translating cells in the presence of phosphate plus D-glucose, or arsenate plus PMS, ascorbate appeared as streaks or tracks. Cells suspended in arsenate plus D-glucose appeared as blurs (but not tracks) as a result of incessant flexing. Immotile cells appeared as grossly overexposed, but relatively still images which can easily be discerned from the blurred images of flexing cells.

Relationship between proton motive force and motility in *S. aurantia.* The proton motive force (Δp) can be defined as the work per unit charge required to move a proton from the outside of a cell to the inside (18, 19, 37, 38). The Δp consists of two components, the electrical potential between the inside and outside of the cell ($\Delta\psi$) and the pH difference between the inside and outside of the cell (ΔpH), as indicated by the equation $\Delta p = \Delta\psi - Z\,\Delta pH$, where Z is the factor used to convert pH to millivolts (18, 19).

CCCP and 1799 conduct protons across biological membranes (18, 43), and as described above these compounds rapidly abolish motility in *S. aurantia.* This suggests that motility in this spirochete requires a proton motive force or some component of a proton motive force (Δp, $\Delta\psi$, or ΔpH). Valinomycin conducts potassium ions across biological membranes (20). As indicated by changes in fluorescence of Di-S-C_3(5), valinomycin dissipated $\Delta\psi$ in metabolizing cells in potassium phosphate buffer plus D-glucose, but had little effect on $\Delta\psi$ of cells in sodium phosphate buffer plus D-glucose (Fig. 3). As indicated by changes in fluorescence of 9-aminoacridine, valinomycin had little effect on ΔpH of *S. aurantia* in potassium or sodium phosphate buffer plus D-glucose (Fig. 4). Nigericin exchanges protons for potassium ions (20). As indicated by changes in the fluorescence of Di-S-C_3(5), and 9-aminoacridine, nigericin dissipated ΔpH but not $\Delta\psi$ of cells suspended in potassium phosphate buffer plus D-glucose. Nigericin did not dissipate either ΔpH or $\Delta\psi$ of cells in sodium phosphate buffer plus D-glucose (Fig. 3 and 4). The separate addition of valinomycin or nigericin to cells suspended in potassium phosphate buffer plus D-glucose did not abolish motility; neither did the addition of both valinomycin and nigericin to cells suspended in sodium phosphate buffer plus D-glucose abolish motility. However, when nigericin and valinomycin were simultaneously added to cells in potassium phosphate

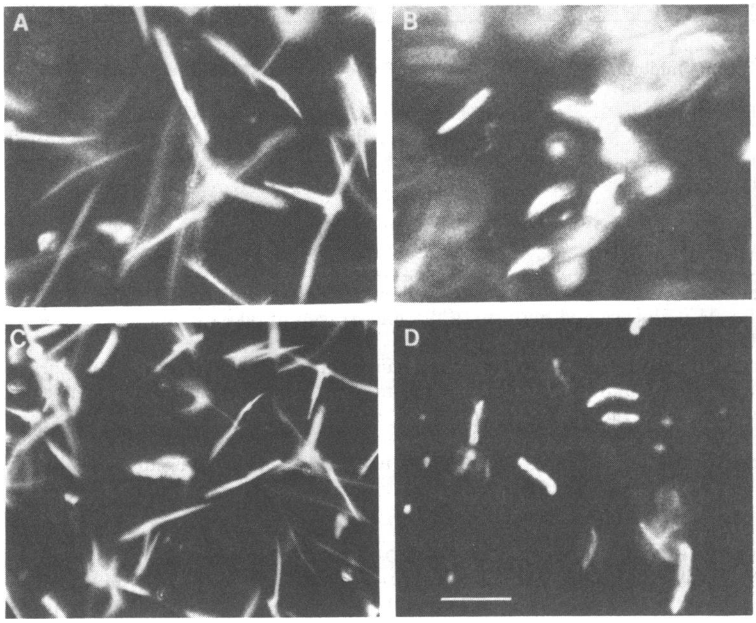

FIG. 2. *Dark-field photomicrographs showing effects of various treatments on motility of S. aurantia. Cells were incubated in (A) potassium phosphate buffer plus D-glucose, (B) sodium arsenate buffer plus D-glucose, (C) sodium arsenate buffer plus PMS–ascorbate, and (D) potassium phosphate buffer plus D-glucose and 1799 (10 μM). Photomicrographs (3-s exposure time) were taken after an incubation time of 15 min in each buffer. Marker bar equals 20 μm.*

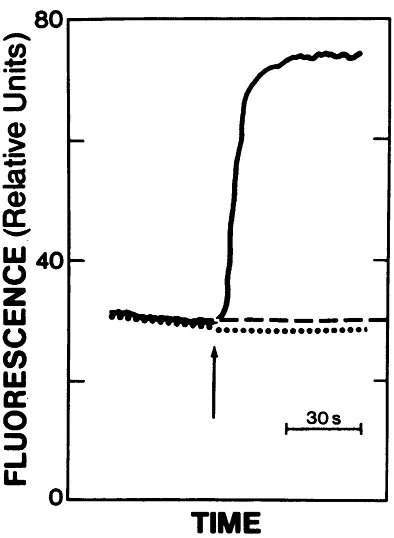

FIG. 3. *Fluorescence of Di-S-C_3(5) in suspensions of S. aurantia. Arrow indicates time valinomycin (50 nM) was added to cells suspended in potassium phosphate buffer plus D-glucose (—) or cells suspended in sodium phosphate buffer plus D-glucose (····), or the time of nigericin (1 μM) addition to cells in either buffer (---). A fluorescence intensity of 32 U corresponded to a negative membrane potential of greater than −90 mV, and a fluorescence intensity of 80 U corresponded to a negative membrane potential of −10 mV.*

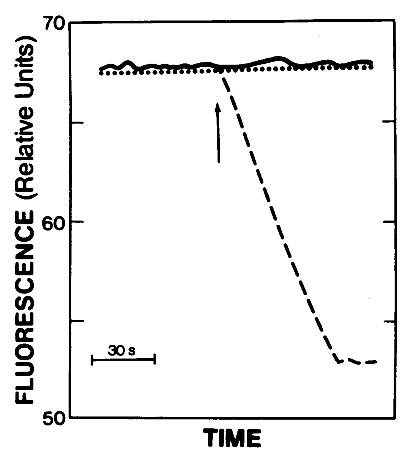

FIG. 4. *Fluorescence of 9-aminoacridine in suspensions of S. aurantia. Arrow indicates time of nigericin (1 μM) addition to cells suspended in potassium phosphate buffer plus D-glucose (----) or cells suspended in sodium phosphate plus D-glucose (—), or time of valinomycin (50 nM) addition to cells in potassium phosphate buffer plus D-glucose (····). When sodium phosphate was used in place of potassium phosphate the effect of valinomycin was essentially as shown. A fluorescence intensity of 68 U corresponded to a ZΔpH of 40 mV and a fluorescence intensity of 53 U corresponded to ZΔpH of <5 mV.*

TABLE 2. *Effects of valinomycin and nigericin on motility of S. aurantia*[a]

Antibiotic added[b]	Buffer[c]	% Motile	Avg velocity (μm/s)
None	Potassium phosphate	95	28
None	Sodium phosphate	95	27
Valinomycin	Potassium phosphate	90	24
Valinomycin	Sodium phosphate	95	24
Nigericin	Potassium phosphate	90	17
Nigericin	Sodium phosphate	95	27
Valinomycin-nigericin	Potassium phosphate	0	0
Valinomycin-nigericin	Sodium phosphate	85	18

[a] Motility was measured after 1 min of incubation in the presence of valinomycin or nigericin or both.

[b] Valinomycin was added to a final concentration of 50 nM, and nigericin was added to a final concentration of 1 μM.

[c] Cells were suspended in either sodium or potassium phosphate buffer (10 mM, pH 7.0), as indicated, plus 5 mM D-glucose.

TABLE 3. *Relationship between pH of suspending buffer, ΔpH, ΔΨ, and motility in S. aurantia*

pH of suspending buffer[a]	Nigericin-induced quenching of 9-aminoacridine fluorescence (relative units)[b]	% Motile[c]	
		−Valinomycin	+Valinomycin
6.5	18	95	95
6.6	14	95	95
6.8	12	95	95
7.0	12	95	95
7.2	10	95	95
7.4	8	95	90
7.6	6	95	90 (flexing)[d]
7.7	1	95	50 (flexing)[d]
7.8	2	95	10 (flexing)[d]
7.9	0	95	0

[a] Cells were suspended in 10 mM potassium phosphate.

[b] Nigericin was at a final concentration of 1 μM.

[c] Determined after 1 min of incubation in the presence or absence of 50 nM valinomycin. Unless otherwise specified, translating cells were predominant.

[d] Flexing was the predominant behavior of motile cells as indicated.

buffer plus D-glucose, motility was rapidly inhibited (Table 2). This indicates that either $\Delta\psi$ or ΔpH can support motility in *S. aurantia* and if both $\Delta\psi$ and ΔpH are dissipated, cells are rendered immotile.

When nigericin was added to cell suspensions in potassium phosphate plus D-glucose, the fluorescence of 9-aminoacridine was quenched to a decreasing extent as the buffer pH was increased over a range of 6.5 to 7.6. At pH values between 7.7 and 7.9, the addition of nigericin resulted in little or no quenching of 9-aminoacridine fluorescence (Table 3). This indicated that as the pH external to the cells increased, ΔpH (outside acid) of *S. aurantia* decreased. At the high external pH values (7.7 to 7.9), ΔpH was not detected. However, cells remained motile over the entire pH range of 6.5 to 7.9 (Table 3) in the presence or absence of nigericin. This supports the conclusion that ΔpH was not required for motility of *S. aurantia*. Presumably, $\Delta\psi$ supported motility when the pH of the suspending buffer was 7.7 or higher. This was demonstrated by the dissipating $\Delta\psi$ of cells suspended in buffers at various pH values by adding valinomycin and then monitoring motility. Translational motility occurred at pH values of 6.5 to 7.4 (relatively high ΔpH) in the presence of valinomycin. When the pH was 7.5 or 7.6, most cells remained motile in the presence of valinomycin but flexed incessantly. When the pH was above 7.6 (and ΔpH could not be detected) valinomycin rendered a large percentage of cells immotile (Table 3).

DISCUSSION

The findings presented in this paper indicate that Δp and not ATP is required for motility in *Spirochaeta aurantia*. In the presence of arsenate plus an electron donor, cells contained little ATP but were motile (Fig. 1 and Table 1) and in the presence of proton ionophores, cells contained much higher levels of ATP yet were immotile (Table 1). When the $\Delta\psi$ component of Δp was dissipated by addition of valinomycin (Fig. 3), cells remained motile so long as the ΔpH component was not dissipated (Table 2, Fig. 4). Thus, $\Delta\psi$ did not appear to be required for motility. If the ΔpH component was dissipated by addition of nigericin (Fig. 4) or by increasing the pH of the external buffer (Table 3), cells remained motile so long as the $\Delta\psi$ was not dissipated (Fig. 4, Tables 2 and 3). Apparently, ΔpH was not required for motility. However, cells were immotile when both $\Delta\psi$ and ΔpH were dissipated (Tables 2 and 3). This indicates that a Δp in the form of $\Delta\psi$, ΔpH, or both was required for motility of *S. aurantia*.

To our knowledge this is the first report which demonstrates either $\Delta\psi$ or ΔpH in a spirochete.

It was found that $\Delta\psi$ of *S. aurantia* suspended in potassium phosphate buffer (10 mM, pH 7.0) plus D-glucose was at least −90 mV (cell interior negative), the maximum value that can be measured by the fluorescent dye technique used (54). Cells in the same buffer exhibited a $Z\Delta pH$ of 40 mV (cell interior alkaline). These data indicate that the components of Δp in *S. aurantia* are qualitatively and, to the extent of our knowledge, quantitatively similar to those in most other bacteria in which $\Delta\psi$ and ΔpH have been measured (26).

The surface of *Mixotricha paradoxa*, a protozoan found in guts of certain termite species, is covered with spirochetes attached by one of their cell ends (13). The attached spirochetes have been observed to undulate in a coordinated fashion and serve to propel motile cells of *Mixotricha*. In fact the attached spirochetes were originally mistaken for flagella (52). This has led to the suggestion that a spirochete or spirochete-like organism was the procaryotic ancestor of eucaryotic flagella (33, 34). It might be predicted that an evolutionary relationship between spirochetes and eucaryotic flagella would be reflected by similarities in the mechanisms of spirochetal and eucaryotic flagellar movements. However, movements of eucaryotic flagella are mediated by specific structures called microtubules which are activated by ATP (46, 47, 51) rather than Δp. In contrast, the mechanism of motility in *S. aurantia* appears to be closely related to mechanisms of motility in other bacteria (see below). This suggests that some other type of procaryote may be more closely related than spirochetes to the organism which evolved into eucaryotic flagella, or it may be that eucaryotic flagella do not have endosymbiotic origins. There is no evidence to indicate that the symbiotic association between *M. paradoxa* and spirochetes reflects an ancient evolutionary development.

The information presented here regarding spirochetal motility is in agreement with evidence regarding the source of energy required for motility of flagellated and gliding bacteria. It has been reported that the flagellated, gram-positive bacteria *Bacillus subtilis* and *Streptococcus* V4051 as well as *Rhodospirillum rubrum*, a gram-negative, flagellated bacterium, require a Δp for motility (15, 32, 35, 36, 48). Furthermore, *Escherichia coli* (30), as well as gliding bacteria such as *Flexibacter polymorphus* (44), requires Δp or some component of Δp for motility rather than ATP.

Berg (2) has suggested that the axial fibrils of spirochetes rotate within the periplasmic space to propel the cell in a manner that is somewhat analogous to the rotation of bacterial flagella. The available information is consistent with this hypothesis inasmuch as axial fibrils are chemically and morphologically similar to bacterial flagella (5, 6, 21, 22, 23, 27, 31, 39) and mutants of spirochetes lacking axial fibrils (41) or containing defective axial fibrils (10) are nonmotile. Furthermore, the energy requirements for motility of *S. aurantia* and of flagellated bacteria are similar. Although it remains to be determined whether axial fibrils actually rotate, there appears to be an evolutionary relationship between the distinct motile behaviors of spirochetes and flagellated bacteria.

Recent evidence has led to the suggestion that gliding motility is driven by rotary assemblies located in the cell envelope (42). Although the mechanism of gliding motility is still a matter of conjecture, it may be that the motilities of flagellated bacteria, gliding bacteria, and spirochetes have similar mechanisms, and perhaps these distinct types of procaryotic motilities have a common evolutionary origin.

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