PROTONMOTIVE FORCE SUPPORTS GLIDING IN CYANOBACTERIA

T. N. GLAGOLEVA, A. N. GLAGOLEV*, M. V. GUSEV and K. A. NIKITINA

Department of Plant Physiology, Biological Faculty, Moscow State University, Moscow 117234 and **Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR*

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1. Introduction

Various cyanobacteria display gliding on the surface of solid substances or through viscous media. Gliding is motion without an external locomotive organelle or a visible change in body shape. Cyanobacteria gliding on a solid surface leave a track of slime. It was proposed, therefore, that the organism is driven by the reactive force of extruded slime. However, not all motile cyanobacteria extrude slime [1]. Moreover, simple calculation has shown that a gliding trichome must empty its contents several times per second in order to account for the observed speed (1–10 μm/s) [2]. In 1959, Jarosh proposed that cyanobacteria are wrapped in parallel rows of small helical fibrils [3]. It was suggested that the fibrils propagate helical waves upon contraction as flagella of eucariotes do. In an electron microscopic study of a giant cyanobacterium *Oscillatoria princeps*, the predicted fibrils were discovered [4,5]. In between the rigid cell wall and the outer membrane lay a layer of parallel fibrils 50–80 Å diam. These fibrils were found to be continuous along the trichome, passing over the cell intersections. The helical lattice of fibrils formed a 60° pitch with the trichome long axis. Most blue–green algae rotate while gliding. Any point of the surface of *O. princeps* traces a helix with a 60° pitch.

We have studied the energetics of gliding motion, considering the discovery of $\Delta\bar{\mu}_{H^+}$-linked motility in *Rhodospirillum rubrum* [6,7], *Streptococcus* [8] and *Bacilus subtilis* [9].

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide; PMS, phenasine methosulfate; DCMU, 1,3-dichlorophenyldimethyl urea; TTFB, trefluorotetrachlorobenzimidazole

2. Materials and methods

Phormidium uncinatum was isolated from Lake Baikal. Trichomes were grown in Petri dishes on the surface of 2% agar under cool light illumination at 25°C. The mineral salts medium contained (per liter): 5 g KNO_3, 0.1 g K_2HPO_4, 0.05 g $MgSO_4 \cdot 7\ H_2O$, 5 mg $FeCl_2$, 5 mg ammonium citrate. Portions of agar at the periphery of the dish containing highly motile trichomes were used throughout the study. *Oscillatoria* sp. was from a sulphur spring near Sochi in the Caucasus.

Microscopic observations were made in bright field or negative contrast with an NU (Zeiss, Jena) photomicroscope at 640 X. A small square of agar (4 X 4 mm) was cut out and placed on a microscope slide. The speed of movement was determined by a stopwatch, using an ocular scale. Reagents were dissolved in the mineral salts medium and added directly on top of the agar in 10–20 μl drops. The additions did not change the positions of trichomes on the surface. Each experiment resulted from the observation of an individual trichome. Some experiments were performed under infrared illumination (λ, 800–1200 nm) with an MIK-infrared microscope, LOMO, USSR.

3. Results and discussion

The trichome of *Ph. uncinatum* is ~6 μm wide and up to 3000 μm long and consists of 10–1000 cells. The main speed on agar surface is 3.0 μm/s. When moving the trichome rotates clockwise.

Uncouplers are known to block the movement of various cyanobacteria [10]. This result was always interpreted as a consequence of exhaustion of the

ATP pool due to the uncoupling of membrane (light-driven and oxidative) phosphorylation [10,11]. The initial action of uncouplers is directed to dissipate $\Delta\bar{\mu}_{H^+}$. It seemed interesting to resolve the nature of uncoupler action on motility caused either by lowering ATP or directly by dissipating $\Delta\bar{\mu}_{H^+}$.

Motility was totally arrested upon addition of 10^{-5} M TTFB or 10^{-6} M CCCP in $\leqslant$3 s (time necessary for observation). At these concentrations TTFB and CCCP inhibit mitochondrial oxidative phosphorylation. Uncouplers not only inhibit phosphorylation but may activate the reversible H^+–ATPase. If an H^+–ATPase inhibitor, DCCD, is introduced, then uncouplers cannot change the ATP concentration. When added to *Ph. uncinatum* DCCD did not inhibit motility and did not prevent or decrease the rate of uncoupler inhibition. The addition of 10^{-4} M DCCD to trichomes respiring in the dark caused a 2–3-fold decrease in ATP level and prevented light-induced ATP synthesis (not shown).

Cyanobacteria may form $\Delta\bar{\mu}_{H^+}$ in non-cyclic electron flow through photosystems I + II; cyclic electron flow at the level of photosystem I; during respiration, and probably upon ATP hydrolysis by H^+–ATPase. If gliding were $\Delta\bar{\mu}_{H^+}$-dependent we might expect any single proton-pump to support motility. To test this assumption an inhibitor analysis was performed. DCCD was used to block the H^+–ATPase. Cyanid inhibits respiration and non-cyclic electron transport at the level of plastoquinone [10]; DCMU inhibits oxidation of H_2O by photosystem II, and PMS and ferrocyanide oxidize electron carriers of photosystem I.

Respiration and cyclic and non-cyclic electron transport support motility in the presence of DCCD (table 1). Gliding was absent when ATPase was the only operating proton pump. This could be due to several reasons:

(i) The absence of a sufficient ATP pool;
(ii) The inhibiting action of a protein inhibitor;
(iii) A lack of sufficient quantities of ATPase in the cytoplasmic membrane.

Motility of a 12 h culture ceased in 30–50 s in the dark. Therefore, a respiratory redox chain is probably absent in the cytoplasmic membrane of these trichomes. The introduction of cyanide to older cultures arrested motility in ~1 min. Motility was immediately restored upon illumination.

The ionophores valinomycin and nigerycin produced an uncoupling effect on cyanobacteria *Plectonema* and *Anacystis* [12]. The addition of valinomycin and nigerycin to an *Oscillatoria* sp. from a sulphur spring totally inhibited motility (table 2). Valinomycin makes cells permeable for K^+ and thus lowers $\Delta\psi$; nigerycin, exchanging K^+ for H^+, dissipates the pH gradient. Valinomycin and nigerycin alone did not inhibit motility (table 2) in the presence or absence

Table 1
Inhibitor analysis of *Ph. uncinatum* motility

Inhibitor	Final conc. (M)	Inhibiting action	
		Young culture	Old culture
TTFB	10^{-5}	(+)	(+)
CCCP	10^{-6}	(+)	(+)
DCCD	10^{-4}	–	–
DCCD +	10^{-4}		
TTFB	10^{-5}	(+)	(+)
CN^-	5×10^{-3}	–	–
DCMU	10^{-5}	–	–
Darkness		(+)	–
CN^- in darkness	5×10^{-3}	(+)	(+)
DCMU +	10^{-5}		
$^+K_3$ Fe(CN)$_6$	5×10^{-3}	–	–
$^+$PMS	10^{-5}		
CN^- +	5×10^{-3}		
$^+K_3$ Fe(CN)$_6$	5×10^{-3}	(+)	(+)
$^+$PMS	10^{-5}		

In the presence of uncouplers, motility was inhibited immediately, and in other cases marked by (+) it ceased in 1 to 3 min. Unless specially indicated, observations were made under bright white light illumination

Table 2
Motility of *Oscillatoria* sp. from a sulfur spring

Inhibitor	Final conc. (M)	Inhibiting action	Inhibiting rate
TTFB	10^{-4}	(+)	Immediately
Valinomycin	2×10^{-5}	–	
Nigericin	10^{-5}	–	
Valinomycin +	2×10^{-5}		
nigericin	10^{-5}	(+)	4–5 min
DCCD	10^{-4}	–	
Valinomycin +	2×10^{-5}		
nigericin	10^{-5}	(+)	4–5 min
DCCD	10^{-4}		
DCMU	10^{-5}	–	
CN^-	5×10^{-3}	(+)	5–10 min

Trichomes were observed under bright white illumination. The main speed in the absence of inhibitors was 3–4 μm/s

of DCCD. This means that gliding is equally well supported by the electric ($\Delta\psi$) or osmotic, ΔpH component of $\Delta\bar{\mu}_{H^+}$. An analogous result was obtained with flagellated bacteria [6].

The effect of an artificially-imposed ΔpH on gliding was studied on de-energized trichomes. Trichomes were kept under dim blue–green illumination at pH 9.0 in the presence of cyanide for 5 min, when a drop of acidic medium was added to bring the pH to 5. The base–acid shift restored the normal gliding rate of trichomes which gradually decreased to zero in ~8–10 min. A typical experiment is shown in fig.1. The same result was obtained in the presence of DCCD, demonstrating that the restoration of motility was not due to an increase of ATP by a ΔpH-dependent synthesis. After the acid-induced motion had ceased (fig.1b), addition of alkali media (pH 9.5) had no effect, indicating that an inversed ΔpH is inactive in supporting gliding.

It is noteworthy to mention that immobilization in the dark after saturating illumination was much more rapid (<2 min) than after imposing an artificial ΔpH (~8 min). Perhaps, in the naturally energized trichomes, $\Delta\bar{\mu}_{H^+}$ is composed mainly of $\Delta\psi$ which rapidly dissipates, due to the small electrical capacity of the membrane. The large artificial ΔpH (3.5) dissipates slowly, due to the significant buffering capacitance of the cytoplasm and the outer media. Owing to the short time span of dark movement after illumination (<2 min), we may also conclude that an H^+–ATPase is not operating to support $\Delta\bar{\mu}_{H^+}$ under these conditions. This conclusion is confirmed by the fact that DCCD does not reduce the time span of motion in the dark after illumination (fig.1a).

In young cultures of *Ph. uncinatum* the addition of valinomycine in the light caused a drastic decrease in the gliding rate and even its complete inhibition (fig.2). After a short lag the speed of gliding gradually resumed its initial meaning. It seems that the initial flux of valinomycin and K^+ into the cytoplasm depolarised the membrane, resulting in a decrease of $\Delta\psi$ and the inhibition of motility. The constant operation of the proton pumps would build up a pH difference, compensating the decrease of $\Delta\bar{\mu}_{H^+}$ and restoring the motility rate.

Gliding upon an artificially imposed membrane potential was studied under photosynthetically inactive infrared illumination. Trichomes were inoculated into a Petri dish with 2% agar; the mineral salts portion was as in section 2, except that K^+ was substituted for Na^+. After gliding in the dark had ceased, valinomycin in a K^+ free medium was added (fig.3a). Trichomes resumed motility that gradually decreased to zero. The addition of valinomycin allowed K^+ to

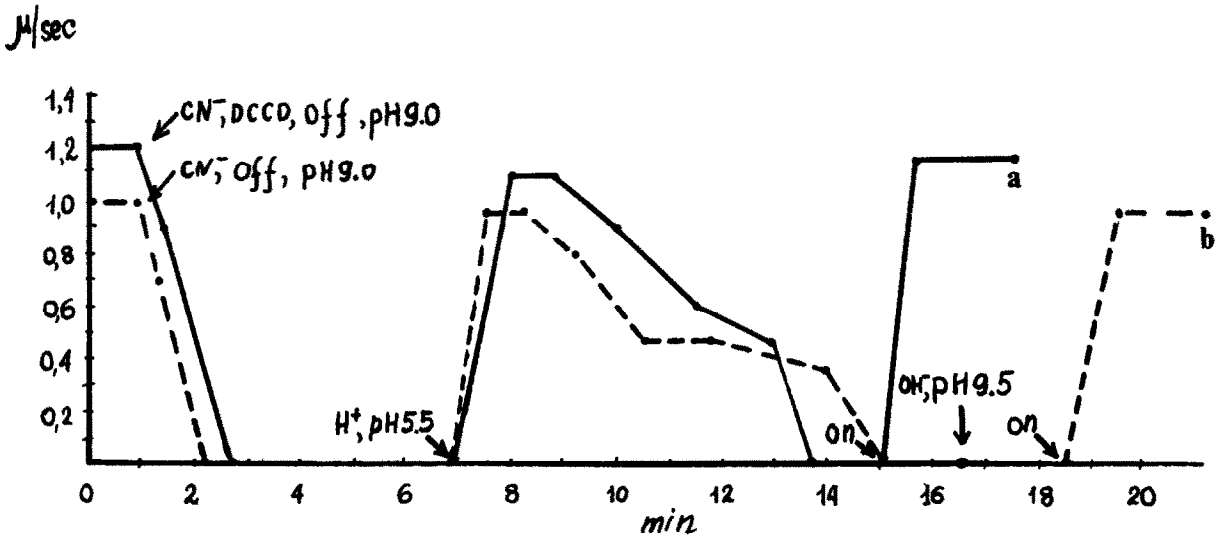

Fig.1. Motility of *Ph. uncinatum* supported by an artificial ΔpH. In both cases behaviour of an individual trichome was observed. (a) (—•—) A trichome on the surface of agar was kept under dim blue–green illumination (off) in the presence of DCCD and CN^-. Addition of acidic medium or illumination by white light(on) are indicated by arrows. (b) (–•–) conditions as in (a) except that DCCD was ommited.

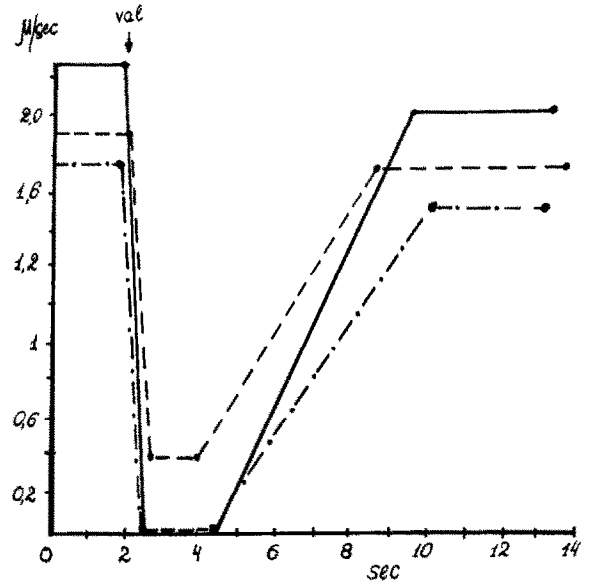

Fig.2. Effect of valinomycin on motility in the light. Three individual trichomes in K^+ medium were illuminated by white light. At times indicated, 10^{-5} M valinomycin was added.

diffuse outwardly down its concentration gradient and to form a membrane potential inside negative. The resulting motility gradually decreased, presumably due to the dissipation of the K^+ gradient. White light restored gliding.

In a control experiment valinomycin in K^+ medium was added to *Ph. uncinatum* cultivated on agar containing a K^+ medium (fig.3b). In this case the K^+ gradient was absent and valinomycin did not cause motility in the dark.

The above data clearly demonstrate that gliding in cyanobacteria is a $\Delta\bar{\mu}_{H^+}$-dependent process, as is motility in flagellated bacteria.

In a study on *Flexibacter polymorphus* [13], the gliding of this unicellular heterotrophic bacterium was found to be inhibited by uncouplers and inhibitors of the redox chain to an extent that could not be accounted for by the observed lowering of cytoplasmic ATP.

It is reasonable to assume that all morphologically related gliding bacteria use the same $\Delta\bar{\mu}_{H^+}$-dependent mechanism and, moreover, share its basic principles with that of flagellated bacteria.

Since the protonmotive force is imposed across the cytoplasmic membrane and we find that gliding is $\Delta\bar{\mu}_{H^+}$-dependent, we would predict that the fibrils of cyanobacteria lying between the peptidoglycin layer and the outer membrane stem from the cytoplasmic membrane. By analogy, it seems probable to assume that the fibrils are attached to the cytoplasmic membrane by a basal body, discovered in flagellated bacteria [14–16]. Transport of H^+ into the cytoplasm through the basal body would cause its rotation [7].

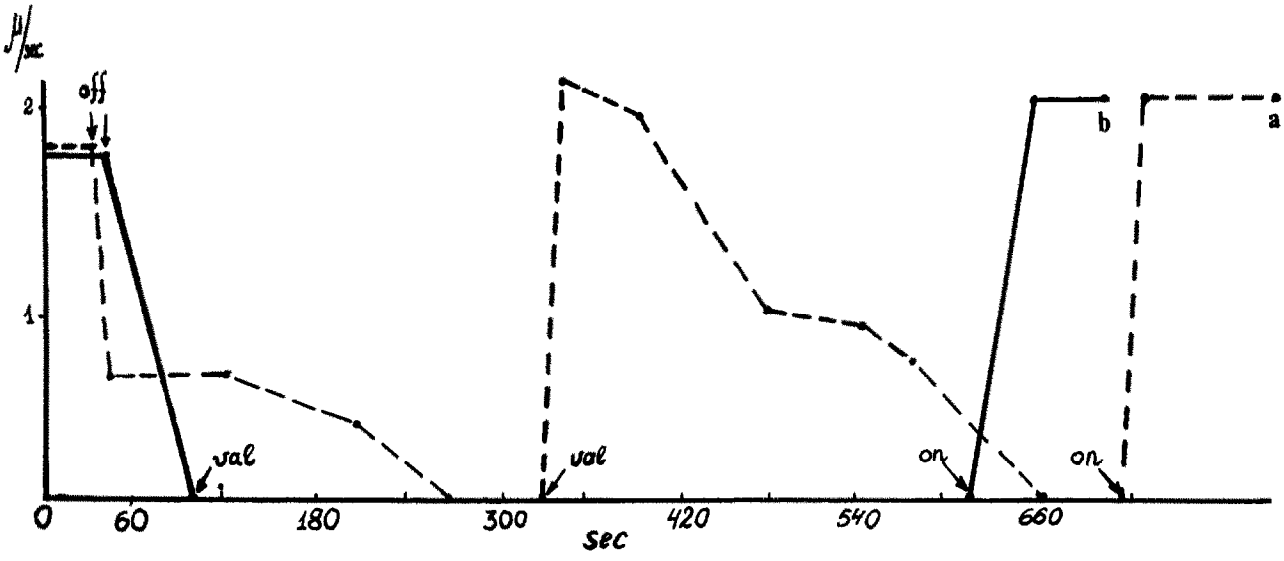

Fig.3. Gliding of *Ph. uncinatum* supported by an artificial $\Delta\psi$. Two individual trichomes were visualized under infrared illumination (off). Valinomycin (10^{-5} M) was introduced at times indicated. At the end of the experiment, white light was turned on. (a) (–•–) Na^+ medium; (b) (—•—) K^+ medium.

The adjacent fibrils, rotating under the elastic outer membrane, would produce a running wave on its surface that would move the trichome against a solid surface.

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