Plant Cell Physiol. 28(4): 587-597 (1987) JSPP © 1987

Energy Supply System for the Gliding Movement of Hormogonia of the Cyanobacterium Nostoc cycadae

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The effects of selected metabolic inhibitors and light on the gliding movement of the hormogonia of the cyanobacterium *Nostoc cycadae* were examined. Respiratory poisons (sodium cyanide, sodium azide) stopped the movement in the dark, but not in light. DCMU had little effect on the movement in light. The inhibitory effect of monoiodoacetic acid (MIA) on the gliding movement in light was restored by adding DCMU, suggesting that the gliding movement in light is linked to a cyclic electron flow involving photosystem I, not to a noncyclic electron flow involving photosystem II. This was then proved by experiments on the effects of selected wavelengths of light on the movement; light absorbed by chlorophyll was found to accelerate the movement, but that absorbed by phycobilins did not.

The proton-conducting uncouplers carbonyl cyanide m-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP) immediately abolished the movement. Arsenate or N,N-dicyclohexylcarbodiimide (DCCD), known inhibitors of ATP synthesis, had little effect on the movement. Light irradiation caused acidification of the cell suspension.

From these results, the direct driving force for the gliding movement of this organism seems to be the trans-cell membrane electrochemical potential difference of protons, generated by a respiratory chain in the dark and a cyclic photoelectron flow system in light.

Key words: Cyclic electron flow — Gliding movement — Hormogonia — Nostoc cycadae — Protonmotive force.

Cyanobacteria (blue-green algae) display a curious "gliding movement", which is an active displacement of the organism which is in contact with a solid material that serves as the structural base on which the motility mechanism of the organism acts without a visible organ responsible for the movement or a visible conformational change in the shape of the organism (Jarosch 1962). Of course, "visible" is defined in terms of the limits of light microscopy and the fact that living material can not be viewed by electron microscopy.

The gliding movements of cyanobacteria have been a subject of research for a long time. An especially well-known type is the "oscillation (swaying)" movement—a type of gliding movement—of the relatively large filamentous cyanobacterium Oscillatoria. Literature dealing with the gliding movements of cyanobacteria spans more than two centuries. Since 1767, many investigators have described gliding movements, discussed their occurrence in nature and offered hypotheses concerning the mechanism responsible for this motility (Halfen 1979). But the mechanism of the gliding movement of cyanobacteria remains unclear.

Halfen and Castenholz (1970, 1971) found a tight, parallel array of 6-9-nm-wide continuous fibrils lying beneath the lipopolysaccharide outer membrane of the giant cyanobacterium

Abbreviations: MIA, monoiodoacetic acid; CCCP, carbonyl cyanide m-chlorophenylhydrazone; DNP, 2,4-dinitrophenol; DCCD, N,N-dicyclohexylcarbodiimide; PIPES, piperazine-N,N-bis(2-ethanesulfonic acid).

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Oscillatoria princeps; they regarded the fibrils as the motile organ, assuming that ATP was probably directly involved in the gliding movement. Since then, however, there have only been a few other significant findings on or explanations of this mechanism in cyanobacteria. Häder (1978) found a correlation between light-induced cell membrane potential changes and photophobic reactions in *Phormidium uncinatum*, and Glagoleva et al. (1980) proposed protonmotive force as an energy source for the gliding movement of *Phormidium uncinatum*.

The present study tried to clarify the energy supply system for the motile organ of the gliding movement of cyanobacteria using Nostocacean hormogonia. The effects of various metabolic inhibitors and light on gliding rate were examined. The effects of those on photosynthetic activity, respiratory activity, the ATP level in the cell, and the pH change of the cell suspension were also determined and compared with those effects on the gliding rate.

Materials and Methods

Isolation and culture of the organisms—In 1977, Nostoc cycadae was aseptically isolated from the coralloid root of the cycad Cycas revoluta (grown in the experimental field of Wakayama University, Wakayama) according to the method of Watanabe and Kiyohara (1963). It was then successively cultured in 9-cm petri dishes on agar plates containing a modified Detmer's medium (Watanabe 1960) under continuous daylight fluorescent lamp illumination of about 0.4 mW · cm⁻² at agar plate level at 20°C. The modified Detmer's medium contained 0.7 mM K₂HPO₄, 1 mM KNO₃, 1 mM MgSO₄ · 7H₂O, 0.5 mM CaCl₂, 1 mM NaCl, 0.015 mM FeSO₄ · 7H₂O, 0.1% Arnon's A₅ microelement solution (Kratz and Myers 1955) and 1.5% agar in distilled water.

Isolation of hormogonial colonies—A segment of the algal mat (about $5 \text{ mm} \times 5 \text{ mm}$) taken from an agar plate of about one month's culture was transferred to 2.5 ml of 1.5 mM phosphate buffer (pH 7.8) containing 1.5 mM MgCl₂ in a flat-bottomed glass tube. After incubation for one night under the culture conditions, many tiny spherical colonies formed at the bottom of the tube. These colonies, composed only of motile hormogonia, were transferred with a micropipet into a glass tube and washed twice with 1.5 mM phosphate buffer (pH 7.8), and then used for the following experiments.

Measurement of gliding rate—To measure the gliding rate, a hormogonial colony of about 200– 300 μ m in diameter was put on the center of the glass plate used to measure the gliding rate and covered with a cover glass (Fig. 2). The space between the plate and the cover glass (100 μ m deep) was filled usually with 1.5 mM phosphate buffer (pH 7.8) containing 1.5 mM MgCl₂ and test reagents. The solution was injected carefully by means of a microsyringe and the radius of the colony was measured with a light microscope. The plate was then kept in a moist chamber of transparent plastic in light (0.4 mW \cdot cm⁻²) or in the dark at 20°C. After a suitable incubation period (usually 1 h), the radius of the expanded hormogonial colony was determined by measuring in four directions with a light microscope. The difference of the radii before and after incubation was regarded as the gliding rate of the hormogonia during the incubation period.

In two cases, to determine the basic experimental conditions—pH (Fig. 3) and metal ions (Fig. 4)—the gliding rate of an individual hormogonium was measured directly by a stopwatch under a light microscope equipped with an eyepiece micrometer and illuminated by an incandescent lamp (Olympus LSE) at about 0.9 mW cm^{-2} fluence rate with a heat-absorbing filter.

Determination of photosynthetic O_2 evolution and respiratory O_2 consumption—Photosynthetic and respiratory activity were measured polarographically with a Clark-type oxygen electrode (Model 5331, YSI Co. Inc., U.S.A.) in a closed cuvette containing 2.8 or 3.0 ml of the cell suspension in a buffer solution (3 mM phosphate buffer or 5 mM Bicine buffer, pH 7.8) containing 2 mM NaHCO₃. The suspension was maintained at 20°C by means of a circulating-water jacket and kept under

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continuous stirring with a magnetic stirrer. To measure the photosynthetic activity, the samples were illuminated with a microscope incandescent lamp through a 10-cm-thick layer of water. Unless otherwise stated, the fluence rate was $9.0 \text{ mW} \cdot \text{cm}^{-2}$ on the surface of the cuvette. In the case of irradiation with a colored light, a 300-W projector lamp or a 500-W xenon lamp (Ushio electric Co.) was used as the light source with colored glass or interference filters and a heat-absorbing filter. The chlorophyll *a* content was determined by the method of Mackinney (1941).

Measurement of pH change of the cell suspension—The pH changes of the cell suspension induced by various treatments were measured with a micro-combination glass electrode (MI-410, Microelectrodes Inc., U.S.A.) added to the oxygen electrode system described above. Cells were suspended in 0.05 mm PIPES-HEPES-Tricine buffer. In this buffer, the pH changed in proportion to the change in the amount of H⁺ added to or subtracted from the solution (between pH 6.2 and 7.8). The signal from a Hitachi-Horiba M-8 pH meter connected to the glass electrode and that from the oxygen electrode were recorded on a Hitachi 561 two-pen recorder.

Determination of ATP level—A hormogonium suspension (0.8 ml) in 1.5 mM phosphate buffer (pH 7.8) containing 1.5 mM MgCl₂ and test reagents was incubated for different periods at 22°C in light (daylight fluorescent lamp illumination, 0.4 mW \cdot cm⁻²) or in the dark. The hormogonia were then killed by adding 0.2 ml of 2.5 M perchloric acid and stirring the mixture vigorously for 30 s. The sample was then immediately neutralized with 3.0 M potassium hydroxide, and 0.1 ml of the supernatant and 0.3 ml of 25 mM HEPES buffer (pH 8.0) were mixed in a test tube. The first peak of light emission after the addition of 0.1 ml of luciferin-luciferase solution to the tube was measured with an Aminko ATP photometer (Strehler and Totter 1952).

Measurement of the effect of selected wavelengths of light on the photokinesis—Monochromatic light of $0.1 \text{ mW} \cdot \text{cm}^{-2}$ fluence rate (obtained from the 1/10 model of the Large Spectrograph at the National Institute for Basic Biology, Okazaki) was used to measure the gliding rate. Experiments were supplemented at several wavelength points with monochromatic lights of the same intensity from a 300-W projector lamp which had passed through interference and heat-absorbing filters. The light intensity was measured using a YSI-Kettering Model 65 radiometer (YSI Co. Inc., U.S.A.).

Chemicals—DCMU, CCCP, and DCCD were dissolved at appropriate concentrations in ethanol and diluted to produce aqueous solutions. The final ethanol concentrations were below 0.03%. Ethanol had no effect on the gliding rate at these concentrations. Arsenate buffer, made of KH₂AsO₄ and Na₂HAsO₄, was adjusted to pH 7.8 and used in combination with 1.5 mM MgCl₂. Other reagents were dissolved in 1.5 mM phosphate buffer (pH 7.8) containing 1.5 mM MgCl₂. When the pH of the solution shifted from 7.8 due to the dissolution of high concentrations of alkaline or acid reagents (e.g. 10 mM NaN₃), the pH was adjusted to 7.8 by the addition of 0.1 M HCl or 0.1 M NaOH.

Results

Behavior of hormogonia—The hormogonium of Nostoc cycadae is a straight trichome about 2.5 μ m wide and up to 300 μ m long. It consists of dozens of cylindrical cells with two conical cells at both terminals and has no heterocyst (Fig. 1). When viewed under a light microscope, it appears to glide smoothly in directions parallel to its axis, like a train.

The algal mat formed on the agar culture plate contained both types of trichomes appearing in the Nostocacean life cycle, i.e., non-motile vegetative trichomes and motile hormogonia. When a segment of the algal mat taken from the surface of the agar plate was transferred to a phosphate buffer (1.5 mM, pH 7.8, containing $1.5 \text{ mM} \text{ MgCl}_2$), motile hormogonia, moving and leaving the mat, became entangled with each other in the liquid and aggregated to form many tiny spherical colonies, which then sank to the bottom of the tube during one-night incubation under 590

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Fig. 1 Hormogonia of Nostoc cycadae. Bar=20 μ m.

the culture conditions. The hormogonia put on the glass plate to measure their gliding rate glided in contact with the plate or the cover glass and the colony expanded in diameter corresponding to its gliding rate.

Examination of the effects of pH, metal salts and light intensity on the gliding rate—To determine the experimental conditions in this study, the effects of pH, metal salts and light intensity on the gliding movement were examined. Burkholder (1933) found that the gliding rate of Oscillatoria formosa was not affected over a pH range of 6.4 to 9.5. Hormogonia of Nostoc cycadae also showed a similar gliding rate over the pH range of 7 to 9 (Fig. 3). No motility occurred below pH 4 or above pH 11.



Fig. 2 Schematic illustration of gliding rate measurement. For details, see text.

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The effects of metal salts are shown in Fig. 4. Every metal solution in distilled water arrested motility almost completely at 200 mM, but the manner of inhibition differed between monovalent and divalent cations. After 30-min incubation in the 200-mM salt solution, dilution by changing the solution to a 2-mM salt solution led to the restoration of motility in the Mg^{2+} or Ca^{2+} -treated cells, but not in the Na⁺- or K⁺-treated cells. The osmotic pressure of these cells is not known, but Dewar and Barber (1973) estimated the internal osmolality of *Anacystis nidulans* to be about 460 mOsm, whereas Walsby (1980) reported a much lower value of 160 mOsm for *Anabeana flos-aquae*. The need for Mg²⁺ for the movement was inferred from experiments on chelator effects: glycoletherdiamine-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA) treatment at pH 7.8 (in which Mg²⁺ is only slightly chelated) had no effect on the gliding movement, whereas EDTA treatment at pH 7.8 (in which Mg²⁺ is chelated) stopped the movement. Based on these experiments, 1.5 mM phsophate buffer (pH 7.8) containing 1.5 mM MgCl₂ was used as the control solution for the following experiments.

Healthy hormogonia glided in the dark at a rate of about $0.6 \ \mu m \cdot s^{-1}$ at 20°C in the control solution on a glass plate. White light irradiation accelerated the gliding rate (photokinesis). The light intensity curves for photokinesis and photosynthetic activity are shown in Fig. 5. Photokinesis reached its maximum rate at an intensity of $0.75 \ mW \cdot cm^{-2}$, at which photosynthetic O_2 evolution activity was 60% of the maximum rate. Photosynthetic O_2 evolution reached 90% of the maximum rate at 2.5 mW $\cdot cm^{-2}$ illumination. Unless otherwise indicated, the fluence rate of $0.4 \ mW \cdot cm^{-2}$ was used to measure the gliding rate in light in the following experiments. This fluence rate was the same as that of the culture conditions; it caused hormogonia to glide at a rate of about $1.0 \ \mu m \cdot s^{-1}$ in the control solution (1.5 mm phosphate buffer, pH 7.8, containing 1.5 mm MgCl₂).



Fig. 3 Effect of pH on the gliding rate in hormogonia of *Nostoc cycadae* moving on a liquid-mounted glass slide. A small piece of algal mat was placed in distilled water mounted on a glass slide. After 30-min incubation in a moist chamber under culture conditions, when many motile hormogonia had left the mat and glided onto the glass surface, the distilled water on the glass slide was exchanged with a pH solution. After another 30-min incubation, the gliding rate of an individual hormogonium was measured under a light microscope using a stopwatch. Each point represents the mean of more than 30 measurements; bars indicate the standard deviation. Ten mM citrate-Na₂HPO₄ buffer (pH < 6), 10 mM Tris-HCl buffer (6 < pH < 9), and 10 mM borax-Na₂CO₃ buffer (9 < pH) were used.

Fig. 4 Effect of metal ions on the gliding rate in hormogonia of *Nostoc cycadae* moving on a liquid-mounted glass slide. Chloride salt solutions of Na^+ , K^+ , Mg^{2+} and Ca^{2+} in distilled water were used. The gliding rate of an individual hormogonium was directly measured as described in the legend to Fig. 3. Each point represents the mean of more than 30 measurements. The standard deviations were less than $\pm 0.12 \ \mu m \cdot s^{-1}$.





Fig. 5 Effect of light intensity on the photokinesis rate (closed circles) and photosynthetic O_2 evolution rate (open circles). A microscope incandescent lamp was used as a light source through a 10-cm-thick layer of water. The photokinesis rate was expressed as the value obtained by subtracting the dark control value from the apparent gliding rate. The dark control value was $0.6 \,\mu m \cdot s^{-1}$; the maximum apparent gliding rate was $1.30 \,\mu m \cdot s^{-1}$. The gliding rate was determined by measuring the radii of the expanded circle of the hormogonial colony after 1-h incubation. The maximum photosynthetic rate was $224 \,\mu mol O_2 \cdot (mg \text{ Chl})^{-1} \cdot h^{-1}$.

Effects of metabolic inhibitors on the gliding rate—The effects of various respiratory and photosynthetic inhibitors on the gliding rate are summarized in Table 1. In the dark, NaCN and NaN₃, inhibitors of the respiratory chain, arrested motility almost completely at 50μ M and 10 mM, respectively, whereas in light, almost all and 50% of the motility remained, respectively, at the same concentrations. Even at 1 mM NaCN, 57% of the control motility remained in light.

DCMU at $10 \,\mu$ M, which completely suppressed photosynthetic O₂ evolution, showed little inhibitory effect on gliding rate. In the dark, DCMU had no effect on the gliding rate. MIA inhibited the gliding movement in light, but only a little in the dark. The inhibition in light was restored to some extent by the addition of DCMU.

These results indicate that the gliding movement in the dark depends on the respiratory chain while that in light depends only on a cyclic photoelectron flow, not on a noncyclic one.

Concentration effectivity curves of CCCP, arsenate and DCCD are shown in Fig. 6. CCCP, a proton-conducting uncoupler, at 10 μ M stopped the gliding movement within a few seconds (observed using a videotape system). Immediate cessation was also observed when 250 μ M DNP (at pH 6.0) was added (data not shown). In contrast, arsenate and DCCD, known inhibitors of ATP synthesis, had little effect on the gliding rate below 20 mM and 50 μ M, respectively. Arsenate at 50 mM stopped the movement (Fig. 6b), but this result cannot be



Fig. 6 Effects of CCCP (a), arsenate (b) and DCCD (c) on the gliding rate in light (open circles) and in the dark (closed circles). The gliding rate was determined as described in the legend to Fig. 5.

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Fig. 7 Change in the ATP level with various treatments. L, in light; D, in the dark. Each point represents the value of duplicate experiments. Double arrows indicate the treatments that caused cessation of the gliding movement.

considered due specifically to arsenate because 50 mm phosphate buffer also stopped the movement (data not shown).

Time courses of the ATP level change caused by various reagents were examined (Fig. 7). Of these treatments, only $50 \,\mu$ M NaCN-dark and $10 \,\mu$ M CCCP treatments stopped the movement. A $50 \,\mu$ M NaCN-dark treatment curve (Fig. 7b) and a $20 \,\mu$ M DCCD curve (Fig. 7d) showed similar patterns. Both exhibited sudden decrease and then gradual recovery of the ATP level. These curves seem to be due to a sudden decrease in the ATP synthesis rate at the start of the treatments and, later, a severe suppression of the ATP consumption rate. But only the NaCN-dark treatment stopped the movement. A similar recovery pattern, though in a shorter period, was observed when the organisms were transferred from light to dark (Fig. 7a). In general, there was no correlation between the gliding rate and the ATP level.

Gliding movement in light—The effects of several selected wavelengths of light on photosynthetic activity (O_2 evolution) and the gliding rate were examined.



Fig. 8 Photosynthetic O_2 evolution curves of *Nostoc cycadae*. Cell densities were between $7-8(\mu g \operatorname{Chl} a) \cdot ml^{-1}$. (a) broad-band red or blue light irradiation. A 500-W xenon lamp was used as the light source. R: red light irradiation obtained through a sharp cut filter and a heat-absorbing filter; half band-width, about 50 nm (600-690 nm). B: blue light irradiation through a blue glass filter; transmission peak, 460 nm; half band-width, about 100 nm. (b) Monochromatc light irradiation. A 300-W projector lamp was used. Wavelengths of 643-705 nm were obtained using interference filters. Half band-widths of the transmitted light were less than 10 nm.

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The photosynthetic O_2 evolution rate of blue light irradiation was very low (Fig. 8a) and, in the red light region, light of 643 nm (near the absorption maximum of phycocyanin) exhibited the highest photosynthetic rate (Fig. 8b). In contrast, as shown in Fig. 9, the gliding rate was highly activated by blue light and, in the red light region, the peak of the gliding rate seemed to be near the absorption maximum of chlorophyll *a*, not phycobilin. These results show that photosynthetic O_2 evolution is activated by phycobilin, and the gliding movement in light is driven by light absorbed by chlorophyll *a*, a cyclic photoelectron flow involving photosystem I—all of which is in agreement with the results for DCMU and MIA treatments (Table 1).

Occurrence of proton efflux—An experiment was conducted to verify the occurrence of H⁺ efflux from a cell (Fig. 10). A cell suspension in 0.05 mM PIPES-HEPES-Tricine buffer containing 1.5 mM MgCl₂ was kept in the dark in a closed cuvette until the dissolved O₂ was completely consumed by respiration. As the O₂ concentration decreased, the pH of the solution decreased due mainly to respiratory CO₂ efflux. The pH was adjusted to around pH 7.5 by adding 0.1 M NaOH intermittently. As shown in Fig. 10, a few minutes after the O₂ had been exhausted, the light was turned on. About 1 min later, when detectable O₂ evolution began, a detectable pH decrease also began. This pH drop is thought to be the result of photochemical H⁺ efflux because, in the O₂ free condition, the probability of acidification due to respiratory CO₂ efflux or photorespiratory organic acid secretion can be excluded. The rate of proton efflux was estimated from the curve to be about 100 protons $\mu m^{-2} \cdot s^{-1}$. DCCD at 50 μ M exhibited little effect on this pH drop (data not shown), which means that DCCD works not in the cell membrane, but in the thylakoid membrane and decreases the cellular ATP level (Fig. 7d). After 30 sec, overlapping photosynthetic CO₂ incorporation raised the pH.

Discussion

The gliding movement of hormogonia of *Nostoc* was examined with new methods of collecting hormogonial colonies and measuring the gliding rate. The gliding movement was dependent on respiration in the dark and on a cyclic photoelectron flow system in light.

Phenomena which depend on a cyclic photoelectron flow have been observed in action spectra; e.g., the gliding rate of *Phormidium uncinatum* in light (Nultsch 1962) and acetylene



Fig. 9 Effects of selected wavelengths of light on the gliding rate. The thick line and the thick broken line indicate the gliding rate under the various wavelengths of light obtained from the 1/10 model of the Spectrograph at the National Institute for Basic Biology, Okazaki, and obtained through interference filters, respectively, at the light intensity of $0.1 \text{ mW} \cdot \text{cm}^{-2}$. The thin line is the absorption spectrum of the cell obtained with a Shimadzu UV-3000 spectrophotometer.

reduction (nitrogenase) activity by intact cells in Anabaena cylindrica (Fay 1970). The curves of these action spectra are similar to that of Fig. 9.

MIA, as an inhibitor of the Calvin cycle, raises the NADPH/NADP⁺ ratio (reduction charge) in the cell and inhibits the cyclic photoelectron flow (Heber 1969). Thus 1 m M IA brought about a decrease in the gliding rate to 13% of the control rate in light. The reduction charge, however, did not increase with the addition of DCMU because of the arrested noncyclic electron flow, therefore, the cyclic electron flow drove the gliding movement to 67% of the control rate (Table 1).

ATP has been assumed to be directly involved in the gliding movement of cyanobacteria. Nultsch (1962) thought that cyclic photophosphorylation was more closely related to the gliding movement of *Phormidium uncinatum* in light, but found no quantitative relation between the cellular ATP content and the movement. Halfen and Castenholz (1971) calculated that *Oscillatoria princeps* may expend 0.2-5% of the energy available from oxidative phosphorylation for the movement.

In 1980, Glagoleva et al. first suggested the possibility of protonmotive force-linked gliding movement of cyanobacteria, based on their study of *Phormidium uncinatum*. By directly measuring the gliding rates of individual trichomes, they demonstrated that a de-energized, nonmoving trichome of *Phormidium uncinatum* in darkness could temporarily recover its motility by means of an artificially imposed protonmotive force. The membrane potential was imposed by valinomycin and K⁺; ΔpH by lowering the medium (containing CN⁻) pH from 9 to 5.5. Here, the effect of ΔpH is questionable because the result may have been due to volatilization of CN⁻ due to the decreased pH.

The present study shows that inhibitors of phosphorylation (arsenate and DCCD) had little effect on the gliding rate (Fig. 6) and there is little correlation between the ATP level (Fig. 7) and the gliding rate. These results suggest that the driving force for the gliding movement is not ATP, but a proton electrochemical potential gradient across the cell membrane (protonmotive force), which is established by a respiratory electron flow system in the cell membrane or a cyclic photoelectron flow system involving photosystem I. The immediate cessation of motility within several seconds brought about by uncouplers, CCCP or DNP, strongly supports this possibility. This immediate cessation could also account for the noninvolvement in the inhibition with ADP or

Chemicals	Relative gliding rate	
	In the dark	In light
Control ^a	65	<u>100 ^b</u>
50 µм NaCN	0	100
1 mм NaCN	0	57
10 mм NaN ₃	0	47
10 µм DCMU	65	94
1 тм МІА	60	13
1 mм MIA, 10 µм DCMU		67

Table 1 Effects of metabolic inhibitors on the gliding rate of the hormogonia ofNostoc cycadae in the dark and in light

The gliding rate was determined by measuring the radii of the expanded hormogonial colony after 1-h incubation. Values are averages of duplicate or triplicate experiments.

^a Control solution contains 1.5 mm phosphate buffer, pH 7.8, and 1.5 mm MgCl₂.

^b Relative gliding rate 100 is 0.97 μ m·s⁻¹. For details, see Materials and Methods.

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Fig. 10 Recording of pH change and oxygen concentration change. Cell density was $11.2(\mu g \operatorname{Chl} a) \cdot \mathrm{ml}^{-1}$. Black and white bars indicate dark and light periods, respectively. Vertical arrows show the start of the pH decrease.

AMP which was expected to increase greatly with the CCCP treatment (Fig. 7c).

Evidence for the existence of an electron transport system on the cell membrane of cyanobacteria has been reported recently (Peschek et al. 1981, Peschek 1983, Craig et al. 1984). However, Omata and Murata (1984) have contested the existence of respiratory and photosystem activity in cell membrane fractions of *Anacystis nidulans*. There is no evidence for the existence of chlorophyll *a* in the cell membrane of the hormogonia, but, the cell membrane of the hormogonia may be connected to the newly formed thylakoid membrane—a connection which has been reported by Lang (1968).

Unlike Nostocacean hormogonia and *Phormidium* (Nultsch 1962), *Oscillatoria princeps* exhibits no photokinesis, that is, it glides at the same rate in the dark and in light (Halfen and Castenholz 1971). This means that the motile organ is driven only by the respiratory electron transport system, probably located on the cell membrane, even in light, and photosystems of this species are neither located on nor linked to the cell membrane.

The occurrence of light-induced H^+ efflux was revealed when photosynthetic CO₂ influx became undetectable (Fig. 10). H^+ efflux from cyanobacterial cells was first investigated by Scholes, Mitchell and Moyle (1969), using Anabaena variabilis. They showed that H^+ efflux occurs when cells kept under conditions allowing neither respiration nor photosynthesis are exposed to light or supplied with O₂. Light-induced H^+ efflux was also confirmed by Masamoto and Nishimura (1977), using *Plectonema boryanum* and *Anacystis nidulans*, and cytoplasmic pH was estimated at 8.5 in light and 7.4 in the dark. Light-induced cell membrane potential change (hyperpolarization) in *Phormidium uncinatum* was observed by Häder (1978).

Raboy and Paden (1978) investigated the active transport of glucose in *Plectonema boryanum*. Glucose transport was greatly reduced in the dark by KCN, and in both light and dark by a proton-conducting uncoupler, carbonyl cyanide-p-trifluoromethoxy-phenylhydrazone (FCCP), but was unaffected by DCCD and DCMU. They concluded that the protomotive force of the cell membrane, which is generated by photosynthetic or respiratory electron transport, drives the glucose transport. The results of the present study agree well with their findings on energetics.

The pH of the external solution affects the viscosity of the mucus surrounding the hormogonia, and the viscosity or adhesiveness of the mucus is believed to affect the gliding rate of the hormogonia. Thus, the effect of pH on the gliding rate (Fig. 3) is not only an expression of the relationship between the driving force and the external pH.

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I wish to express my sincere gratitude to Professor Sitiro Sato of Nara University of Education for his criticism, advice, and encouragement throughout this study. I also wish to thank Professor Masashi Tazawa, Dr. Teruo Shimmen and Dr. Tetsuro Mimura of the Faculty of Science, University of Tokyo for their invaluable discussions and help in the use of the ATP photometer; Professor Shigetoh Miyachi and Dr. Tamiko Oh-hama of the Institute of Applied Microbiology, University of Tokyo for their kind advice and help in the use of the Shimadzu UV-3000 spectrophotometer and various interference filters and lamps; Dr. Masakatsu Watanabe of the National Institute for Basic Biology for his kind help with the use of the Spectrograph; and Professor Mitsuo Nishimura of Kyushu University for kindly reading the manuscript.

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(Received April 14, 1986; Accepted March 12, 1987)