Characteristic structures of Spirulina trichome involved in gliding movement

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Summary As an approach to the elucidation of the mechanism of gliding movement of blue-green algae, Spirulina, the structures of trichomes and individual cells were studied using light and electron microscopes. In the two Spirulina species, S. subsalsa of the tightly coiled trichome and S. platensis of the loosely coiled trichome, the spatial arrangement of thylakoids and the region of contact to the substrate were analyzed by comparison. A characteristic found common to both species was that the locus line of the point of contact to the substrate, which was drawn during the gliding movement, corresponded with the outer edge of thylakoid which was arranged along the long axis of trichome. It raises the possibility that the driving force is generated by proton motive force derived from photosynthesis.

Key words: gliding movement, Spirulina, thylakoid, trichome, motive force.

General introductions to the motility of blue-green algae have been presented twice in the last 20 years (Castenholz, 1973a,b, 1982). However progress in understanding the mechanism for the gliding movement has been limited during this period. Furthermore, during the last eight years there has not been notable advancement in disclosing the mechanism.

Several possible mechanisms for gliding in various prokaryotes have been discussed (Castenholz, 1982). In filamentous blue-green algae, the hypotheses previously presented for the mechanisms were based on the driving forces due to either one of or both of the following components: the forward reaction to the forceful ejection backward of mucilage and the backward push of slime by the waving fibrils located between the plasma membrane and the cell wall.

Glagoleva et al. (1980) first suggested the possibility of proton motive force-linked gliding movement in Phormidium uncinatum and Hirose (1987) reported the possibility that the direct driving force for the gliding movement of Nostoc cycadae was the trans-cell membrane electrochemical potential difference of protons.

Thus the gliding mechanism has been investigated from these two different standpoints, the visual observation of

the structure and the physiological analysis of energy supply.

This paper will contribute to the investigation of the gliding mechanism by considering both standpoints together, as well as by presenting a structural analysis of the gliding mechanism in non-amoebean organism which are encased in stiff cell walls.

Materials and Methods

Spirulina subsalsa (IAM M-183) and Spirulina platensis (IAM M-135) were provided by the Institute of Applied Microbiology of the University of Tokyo and the Microbial Culture Collection at the National Institute for Environmental Studies and were successively cultured in the bacteria-free synthetic media, MA (Ichimura, 1979) and SOT (Ogawa and Terui, 1970) respectively. The cultures were maintained under the conditions of 22°C and 1500 lux illumination with fluorescent lamps (12:12h L:D).

A Hitachi H-300 transmission electron microscope and Hitachi S-430S scanning electron microscope were used for the observation of structures and a time-lapse video system was used for the observation of the motility.

Results

The tightly coiled trichome of Spirulina subsalsa (Fig. 1) with a coil-diameter of 3.0µm and an average coil-length of 50.0µm moved on the substrates along the longitudinal axis of the coil as it rotated. The direction of movement was suddenly changed sometimes by unknown triggering factor, demonstrating that the direction of rotation of this right-handed coil could be reversed from counterclockwise to clockwise and vice versa. Observation by scanning electron microscopy showed no specific structures on the surface of the cells (Figs. 1b and c), though traced mucilage seemed to remain. The transmission electron microscopic observation of a longitudinal section of the coil showed that the nucleoid was located continuously along the plasma membrane closest to the central axis of the coil, while the thylakoid was arranged in the outer area of the coil (Fig. 2a). The inner edge of the thylakoid was anchored in the nucleoid and the outer edge seemed to touch the plasma membrane along the outside of the coil (Fig. 2b). A single cell of the trichome was horseshoe shaped but not flat (Fig. 3) and occupied two-thirds of the pitch of the coil. Distinct septa were seen between two cells (Fig. 2a). Each cell was rigid and the coils were not deformed.

Figs 1-3. The trichome structures of *Spirulina subsalsa*. Fig.1. The trichome appearances. a. The loosely aggregated trichomes observed with a light microscope. x250. b and c. The trichomes observed by scanning electron microscopy. Bars: 5.0 μ m for 1b and 1.0 μ m for 1c. Fig.2. The inner structure of trichome observed by transmission electron microscopy. a. A slightly tangential, longitudinal section of a coil. Bar:1.0 μ m. b. The cross section of a cell showing the arrangement of thylakoids. Bar:0.5 μ m. t:thylakoid, n:nucleoid, s:septum. Fig.3. An illustrated single cell (solid line) as a element of the trichome.

The trichome of Spirulina platensis with a diameter of 5.0 μ m was loosely coiled (Figs. 4a and b). The diameter of the coil was 25.0 μ m and a single trichome had 7 pitches in general. S. platensis moved on a substrate, in a manner similar to S. subsalsa, by the rotation of the coil. The coiled structure was rigid and did not contract. Specific structures such as mucilage releasing pores were not observed, though the surface was not as smooth as that of S. subsalsa (Fig. 4c). The trichome was composed of a series of lopsided drum shaped cells (Fig. 5). The cross section of a cell revealed a central nucleoid and radial thylakoids arranged perpendicularly in the cross section (Fig. 6a). One edge of the thylakoid was anchored along the central nucleoid and the other was seen to be extremely close to the plasma membrane. These thylakoids frequently extended into the neighboring cells, suggesting that the septum was formed passing through the thylakoids (Fig. 6b) when the cell divided.

In the case of each of these two types of coiled trichome, when the coil rotated on the flat surface of a substrate, the point of contact to the substrate was described as a line around the outermost surface of the coil as shown in Fig. 7. In either case, it exactly corresponded to the line of the outer edge of one of the thylakoids (Fig. 8), though the plasma membrane and cell wall lay between them.

When the coil moved along its longitudinal axis on the flat surface of a substrate, the point at which the substrate contacted with the coil remained the same but the point of contact on the coil itself continuously shifted along the locus line (Fig. 9). Actually, the outer edge of one thylakoid turned around the central axis of the coil, keeping contact with the same spot on the substrate with only the plasma membrane and cell wall in between.

No special structures were recognized along the plasma membrane corresponding to the edge of the thylakoid, though the existence of some structure responsible for proton flow, such as bacterial flagella-motor apparatus, had been expected. However, transmission electron microscopic observation revealed characteristic structures on the trichome surface of both species, although their roles were unknown. Electron dense small particles were evenly distributed on the whole surface of the trichome of S. platensis (Figs. 10a and b), while in S. subsalsa, some pores were seen which were closely and evenly arranged on the cell surface located at the inner side of the coil (Fig. 10c).

Discussion

The structural correspondency in Spirulina of an outer edge of thylakoid with the locus line of the point of con-

Figs.4-6. The trichome structures of *Spirulina platensis*. Fig.4. The trichome appearances. a. The loosely aggregated trichomes observed with a light microscope. x35. b and c. The trichomes observed by scanning electron microscopy. Bars: 10.0 μ m for 4b and 5.0 μ m for 4c. Fig.5. An illustrated single cell (solid line) as an element of trichome. Fig.6. The inner structures of a trichome observed by transmission electron microscopy. a. cross section of the trichome. b. A slightly tangential lateral section of a trichome. Bars: 1.0 μ m. t:thylakoid, n:nucleoid, s:septum.

Fig. 7. The locus lines of the point of contact to the substrate around the outermost surface of the coil. The thick lines show the locus lines. a: *S. subsalsa*. b: *S. platensis*. Fig. 8. The edge of a thylakoid corresponding to the locus line in Fig. 7. The thick lines show the edge of the thylakoids. a: *S. subsalsa*. b: *S. platensis*. Fig. 9. The illustration of the moving coil. The two large arrows show the direction of movement of the coil. The small arrows show the direction of movement of the trichome which glides on a point of the substrate (the thick arrow).

tact to substrate raises the possibility that the driving force for gliding movement of blue-green algae is trans-cell membrane electrochemical potential difference of protons which are derived from photosynthesis (Hirose, 1987). According to the above observations in *Spirulina*, the site of propulsion is theoretically only one portion of the trichome surface, although it has not been concluded that a sole site is, in fact, enough to generate the gliding movement of a trichome. An increase in number of propulsion sites on a trichome seems to enhance the activity of its gliding movement, since the trichomes are highly active in the colony: in the colony, as the trichomes interwind, the points of contact to the substrate, which is the surface of any other trichome in this case, increase in number.

In *S.platensis*, the thylakoids are arranged radially and there are many outer edges running along the long axis of the trichome. Since the trichomes of this species are more loosely coiled, these outer edges of the thylakoids are all capable of becoming propulsion sites, when they get tangled with each other in the colony. While in *S.subsalsa*, the thylakoids are mostly parallel to each other and their outer edges run along the outermost region of the coil. This seems reasonable, since the propulsion site is only necessary to locate on the outermost region of the coil, because of its extremely tight coiling. Thus the specific arrangement of thylakoids in either species seems to allow these trichomes to obtain the driving force most effectively.

Fig.10. The structures revealed on the surface of trichomes. a and b. The particles distributed along the whole surface of the trichome of *S.platensis*. c. Evenly arranged pores on the trichome surface of *S.subsalsa* at the inner region of the coil. A tangential section of the trichome. The large granules are seen in cytoplasm. Bars:0.2 μ m. pa:particles, t:thylakoid, pm:plasma-membrane, po:pore.

The location of the nucleoids in the trichomes is also different in these two organisms. This discrepancy can be explained reasonably. When the tight coiling of the trichome of *S. subsalsa* became loose in the process of evolution, the nucleoids transferred from the center of the coil to the center of the trichome and the thylakoids changed their distribution from the outer area of the coil to the outer area of the trichome as seen in the structure of *S. platensis*. Or perhaps, when the loose coiling of the trichomes of *S. platensis* became tight, the nucleoid and thylakoids in this trichome must have shifted conversely.

The fine structures on the cell surfaces in each species are under investigation concerning their specific participation in the gliding mechanism.

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滑走運動にかかわるスピルリナ糸状体の特異形態 石川依久子（東京学芸大・生物）

らん藻スピルリナに共通するねじ巻き滑走運動の機作を探る第一歩として、形態の異なる二種のスピルリナの細胞構造を比較し、運動と形態のかかわり合いを考察した。二種に共通の知見として、チラコイドは、トリコームの長軸に沿って長く伸び、チラコイドが細胞と垂直に交わる線上に基質との接触部位があることがわかった。滑走運動にあたって、基質に対する反発力すなわちトリコームの回転を促す推進力（の一部）が光合成由来のプロトンの流れによる（広瀬 1987）ものとすれば、兩種にみられる特異なチラコイド配列はもっとも効率よく運動力を得るための構造として理解される。

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