Short communication

cAMP-triggered *Spirulina* Cell Aggregates Maintain the Shapes of Their Vessels

KAZUKO OHMORI^{*1}, MASAKI HIROSE² and MASAYUKI OHMORI³

¹ Faculty of Life Sciences, Showa Women's University,
Taishido 1-7, Setagaya-ku Tokyo, 154-0004 Japan

² Faculty of Education, Wakayama University, Sakaedani 930, Wakayama-shi, 640-8441 Japan

³ Department of Life Sciences, Graduate School of Arts and Sciences,
University of Tokyo, Komaba, Meguro-ku, Tokyo, 153-0041 Japan

(Received November 10, 1997—Accepted January 23, 1998)

Key Words: *Spirulina platensis*, Cyanobacteria, cAMP, Mat, Aggregate

It is known that some species of cyanobacteria form algal mats or cell aggregates under certain physiological or ecological conditions^{1,2,9}). We found that a filamentous gliding cyanobacterium, *Spirulina platensis*, formed algal mats when cAMP was added to dense cell suspensions⁵). Cyclic AMP was able to trigger mat formation at concentrations as low as 10^{-6} M. Other nucleotides such as cGMP, AMP and ATP had no such effect. It was also found that cAMP stimulated the respiration and gliding motility of *Spirulina* cells^{5,6,7}). Since *Spirulina* cells are known to excrete cAMP into their surrounding medium under routine culture conditions⁸), the cAMP-triggered mat formation would have an ecological significance for the survival of *Spirulina* cells. For example, under certain critical conditions, such as drying up of the habitat, cAMP in the water would be condensed, thus stimulating mat formation. The resulting thick layer of algal cells would enable some *Spirulina* cells to survive in such dehydrated environments.

In the course of our experiments, it was noted that the shapes of cAMP-induced mats, though reduced in size, resembled those of the vessels in which *Spirulina* cells were suspended. In the present paper, we report

that *Spirulina* cells formed aggregates showing exactly the same shape as the vessel. A mechanism explaining how cells take similar shapes to their vessels was determined by using diluted *Spirulina* cell suspensions.

The cyanobacterium *Spirulina platensis* (IAM 135 from the Institute of Applied Microbiology, University of Tokyo) was grown in an SOT medium⁴) containing a high amount of NaHCO_3 (200 mM). The culturing was carried out at 25°C for one week under continuous white light illumination of $52 \mu\text{mol m}^{-2}\text{s}^{-1}$. The culture was bubbled with air. The cells were harvested by filtration with a Toyo No.2 filter paper and suspended in fresh SOT medium at an appropriate concentration. Experiments were carried out at room temperature (about 25°C) and dim white light (less than $3 \mu\text{mol m}^{-2}\text{s}^{-1}$). Chlorophyll *a* concentrations were determined in accordance with the method of Mackinney³) .

Filaments of *Spirulina* were randomly dispersed in their vessels in the absence of exogenously added cAMP during the initial phase of the experimental periods. When 2×10^{-5} M cAMP was added to a dense suspension of filaments ($1.3 \text{ mg Chl ml}^{-1}$), the filaments began to aggregate, and formed a floating mat within 30 min. The shape of the mat was similar, though reduced in size, to that of the vessel which contained the

* Corresponding author; Tel: 03-3411-5111 ext. 2073

filaments (Fig. 1): a smaller round shape in a round vessel (A), a smaller square shape in a square vessel (B), a smaller heart shape in a heart-shaped vessel (C), and such similarity also occurred in vessels with more complicated shapes (D, E). Since the filaments aggregated very tightly, once the mat formed they were not easily dispersed by rocking the vessels.

When *Spirulina* filaments were suspended in a long spectrophotometer cell (W 10 mm $\times$ D 50 mm $\times$ H 50 mm), the filaments formed a vertically thick floating mat (F). This result shows that aggregation and shape formation proceeded three dimensionally in the water. This three dimensional contraction may rule out the possible involvement of surface tension at the glass-air-water interface as a force in the formation of the mat shape.

In order to microscopically determine the process of

aggregation in forming such shapes, aggregation was induced using a more dilute suspension (13 μ g Chl/ml) (Fig. 2). The filaments of less dense suspensions of *Spirulina* were also distributed randomly in the absence of exogenously added cAMP (A) during the experimental periods (over 2 hr). When cAMP (2×10^{-4} M) was added to the suspension in a round dish (30 mm in diameter), the filaments began to glide as rapidly as in the dense suspensions. However, filaments first formed asterisk-shaped aggregates, which was different from the case of dense suspensions (B). These small asterisk-shaped aggregates then connected with each other by forming bridges and then gradually formed a network containing many clusters of filaments (C). The network then contracted, by gliding, to fill the open spaces and at the same time to increase the tightness of the aggregation. As a result of the gliding contraction, the periphery of the network became round, similar to the round shape of the vessel (D). Curiously, the filaments which had been located in the central part of the suspension were pulled towards the periphery and the network became like a wreath (D). A similar process was observed when 2×10^{-5} M cAMP was added to the cell suspension. These observations indicate that neither mat formation nor shape construction is brought about by simple aggregation of filaments in a mechanism that resembles contraction of agar gel during drying. Moreover, these observations suggest that thick disks, as shown in Fig. 1, were only able to be formed only when the densities of the suspensions were high enough to fill the central part of the wreath.

To make the shape of the algal aggregate similar to that of the vessel, it is required that the ratio of the length from the center to the periphery of the aggregate (a' , a'' , b' , b'' in Fig. 3) and those of the vessel (a , b in Fig. 3) should be equal ($a'/a = b'/b$, $a''/a = b''/b$) after certain periods. In order to fulfill this requirement, all of the randomly distributed open spaces in the suspension at the beginning of aggregation, as shown in Fig. 2c, should then shrink at the same rate. It should be noted that the absolute speeds of the mass contractions along each axis are dependent upon the number of filaments or number of open spaces along each axis (Fig. 3). It can be assumed that

Fig. 1. Shapes of mats of *Spirulina platensis* formed by the addition of cAMP in vessels with different shapes. Cell density: 1.3 mg chlorophyll ml^{-1} . The depth of each suspension was about 4 mm. cAMP concentration: 2×10^{-5} M. Pictures were taken from the top (A, B, C, D, E) and from the side (F). In picture (F), the surface of water is indicated by an arrow. Scale bars indicate 20 mm (A, B, C, D, F) and 10 mm (E).

Fig. 2. The formation of an aggregate by *Spirulina platensis* filaments with a dilute cell suspension in a round dish. Cell density: $13 \mu\text{g chlorophyll ml}^{-1}$. (A): without cAMP. Other photographs were taken at 20 min (B), 40 min (C) and 90 min (D) after the addition of cAMP ($2 \times 10^{-4} \text{ M}$). Scale bars indicate 0.1 mm (A,B), 0.2 mm (C) and 1 mm (D).

the final sizes of the mats are proportional to the sizes of the vessel, so that larger mats are formed in larger vessels and smaller mats are formed in smaller vessels. Although this assumption can be applied to the vertical contraction, buoyancy of the cells should be taken into account.

To examine the above hypothesis, the correlation between the sizes of vessels and those of algal mats was studied at various time points. *Spirulina* filaments were suspended evenly in round dishes with different diameters and cAMP was added to all dishes at $2 \times 10^{-5} \text{ M}$. Fig. 4 shows the time course of contraction of the cell aggregates. The ratios of diameters of cell aggregates to those of the vessels were about the same at each time point (about 0.7 and 0.5 at 10 and 60 min, respectively). This observation confirms the above

assumption.

To maintain the original vessel shape during contraction of the aggregate, the adherent force of the filaments should be equal, otherwise, the same gliding speeds cannot be expected, and thus proportional contraction will not be accomplished. It also seems important for proportional contraction that the enhancements of gliding speed last a certain period of time under a certain cAMP concentration. In cases where these necessary conditions are satisfied, *Spirulina* filaments were able to aggregate in proportion to their initial shape before aggregation i.e. the shape of the vessel.

Many unknown factors are likely to be required for shape formation by *Spirulina* aggregates. The physical or chemical property of the wall of the vessel would

Fig. 3. A hypothetical mechanism of mat formation. Open spaces evenly dispersed within the net structure condensed at the same velocity and thus the initial shape of the mat was maintained with a smaller size after a certain incubation period. $t = T_0$: just before the addition of cAMP. $t = T_1$, T_2 : after given periods of incubation in the presence of cAMP. a : the length of the long axis of the vessel, b : the length of the short axis of the vessel. a' , a'' : the lengths of the long axes of the mat, b' , b'' : the lengths of the short axes of the mat. For detail see text.

be expected to affect the final shape of the aggregate. The vessel composition, whether glass or plastic, however, did not affect the shape of the algal mat. Does cAMP only enhance gliding velocities and adherent forces of the filaments, or does it have a more complicated effect on cell aggregation? How do the

Fig. 4. Changes in the diameters of algal disks of *Spirulina platensis* suspended in round dishes of different diameters after the addition of cAMP. Cell density: 1.3 mg chlorophyll ml^{-1} . cAMP concentration: 2×10^{-5} M. Diameter of vessels: 41 mm (●), 35 mm (▲) and 29 mm (■).

gliding rates of filaments affect the formation of the shape? Elucidation of this mysterious phenomenon may lead us to a new biological research theme, namely, recognition by cells of the shape of their surroundings, or shape formation by masses of cells.

Acknowledgment

We thank Dr. Y. Inoue, the Institute of Physical and Chemical Research, for his valuable discussion, and Dr. M. Watanabe, the National Institute for Basic Biology and Dr. F.G. Plumley, University of Alaska, for their critical reviews of the manuscript. This work was supported by a Grant-in-Aid for General Scientific Research (07404045) to M.O. from the Ministry of Education, Science, Culture and Sport, Japan.

References

- 1) Castenholz, R.W. 1967. Aggregation in a thermophilic *Oscillatoria*. *Nature* **215**: 1285–1286.
- 2) Castenholz, R.W. Motility and Taxes, in "The Biology of Cyanobacteria", ed. by N.G. Carr and B.A. Whitton. University of California Press, Berkeley and Los Angeles, 1982, pp.413–439.
- 3) Mackinney, G. 1941 Absorption of light by chlorophyll solutions. *J. Biol. Chem.* **140**: 315–322.
- 4) Ogawa, T. and G. Terui. 1970. Studies on the growth of *Spirulina platensis*: (I) On the pure culture of *Spirulina platensis*. *J. Ferment. Technol.* **48**: 361–367.
- 5) Ohmori, K., M. Hirose and M. Ohmori. 1992. Function of cAMP as a mat-forming factor in the cyanobacterium *Spirulina platensis*. *Plant Cell Physiol.* **33**: 21–25.
- 6) Ohmori, K., M. Hirose and M. Ohmori. 1993. An increase in the intracellular concentration of cAMP triggers formation of an algal mat by the cyanobacterium *Spirulina platensis*. *Plant Cell Physiol.* **34**: 169–171.
- 7) Ohmori, K. and M. Ohmori. 1993. Effect of cAMP on cellular ATP concentration in the cyanobacterium *Spirulina platensis*. *J. Gen. Appl. Microbiol.* **39**: 247–250.
- 8) Sakamoto, T., N. Murata and M. Ohmori. 1991. The concentration of cyclic AMP and adenylate cyclase activity in cyanobacteria. *Plant Cell Physiol.*, **32**, 581–584.
- 9) Walsby, A.E. 1968. Mucilage secretion and the movements of blue-green algae. *Protoplasma* **65**: 223–238.