

PHYCOBILISOMES OF THE THERMOPHILIC BLUE-GREEN ALGA *SYNECHOCOCCUS LIVIDUS*

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Aggregates containing both phycoerythrin and phycocyanin were first described in the red alga *Porphyridium cruentum* and named phycobilisomes (7, 8). This and subsequent investigations of phycobilisomes in blue-green and red algae were carried out in laboratory cultures (6, 9–11, 13). To ascertain if phycobilisomes occur in blue-green algae grown under natural conditions and to compare their structural characteristics with those of laboratory cultures, the unicellular thermophile *Synechococcus lividus*, a natural inhabitant of the hot springs at the Yellowstone National Park, was examined. As reported by Brock (4), this is to date the only known photosynthetic organism growing at temperatures above 60°C. Phycobilisomes were found to be not only abundant in this material, but their structure was more clearly defined than that of mesophilic blue-greens, or of the same thermophilic species cultured in the laboratory. A diagram showing the phycobilisome arrangement in this alga is presented, and its possible structural composition is discussed.

MATERIALS AND METHODS

Cells of *Synechococcus lividus* Copeland, with a vivid blue-green color, were collected at several effluents

from hot springs (pH 8.2–8.4) in the lower Geyser Basin area of the Yellowstone National Park. The temperature of the water at these sites was in the range 65°–70°C. The selected unicells were usually found in shallow, fast-running water, on the sides or bottom of gravel pieces, or in depressions of the rocky edges of the effluents, generally under diffuse light. Special care was exercised to avoid yellowish cells common on sites completely exposed to intense direct sunlight. As reported by Brock and Brock (5), this alga was always associated with extensive bacterial mats.

Cells were fixed *in situ* with a 3% solution of glutaraldehyde in 0.1 M sodium cacodylate by a 1:1 dilution of 0.2 M stock solution, pH 7.0, with filtered pool water. After the cells had settled to the bottom of the test tube (about 2 hr), the supernatant was replaced by cooled filtered pool water. The tubes were then sealed and transported to the laboratory and stored at 4°C for several days. After further washing in the pool water, the samples were postfixed in 1% OsO₄ solution made with pool water and embedded in agar. The agar blocks were dehydrated in a graded series of ethanol-water solutions followed by propylene oxide, and infiltrated with Epon 812. Thin sections were stained with ethanolic uranyl acetate and lead citrate solutions, and photographed in a Siemens Elmiskop IA.

OBSERVATIONS

Phycobilisomes were readily observed in *S. lividus* cells collected from the hot springs. As can be seen in Fig. 1, the phycobilisomes are located in rows parallel to one another on the stroma or outer

side of each photosynthetic lamella. The distance from the center of one row of phycobilisomes to that of the adjacent row is 400–500 Å. The shape of the phycobilisomes depends on the plane of section from which they are viewed. Three planes



FIGURE 1 This slightly oblique section of the thermophilic blue-green alga *Synechococcus lividus* displays various planes of section of the concentrically arranged photosynthetic lamellae and of the phycobilisomes attached to them. In grazing sections (A), the phycobilisomes appear as short dense lines. They have a similar appearance in longitudinal section (B), except that their attachment to the lamellae is visible. In cross-section (C), they have a broad rounded face. $\times 60,000$.

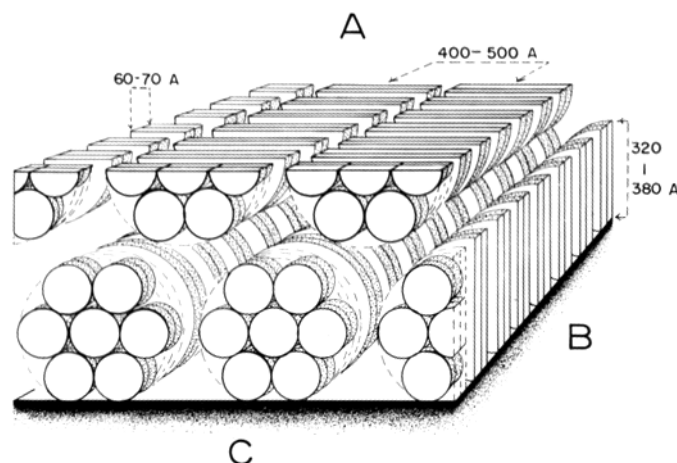


FIGURE 2 A schematic representation showing three views of phycobilisomes such as can be seen in a blue-green alga containing phycocyanin. Face *A* represents a grazing section through the phycobilisome rows but without passing through a membrane. A longitudinal section, in line with the phycobilisome row, is indicated in face *B*. The phycobilisome shape is similar to that in grazing section, except that in this plane the attachment to the lamellae is visible. In cross-section *C*, the phycobilisomes have a round broad face. Also represented is a possible subunit arrangement (plain circles) discussed in the text.

of section of phycobilisome rows are indicated in Fig. 1. In a grazing section over the rows designated *A*, the membrane to which they are attached is out of view but can be presumed to be present above or below the page. In a longitudinal cut indicated by *B*, the phycobilisomes, as in the grazing section, appear as short dense lines, but in this case their attachment to the underlying photosynthetic membrane can be seen. When the plane of section passes vertically across the phycobilisome rows, represented by *C*, they evidence a broad round face. Since they appear round in only one plane and as dense lines in two planes, they are considered to be disc shaped. On the average these disc-shaped phycobilisomes have a diameter of 320–380 A, and a thickness of 60–70 A.

Phycobilisomes definitely tend to occur in rows parallel to one another, but the orientation of the rows is not exclusively with either the long or the short axis of the cell. From freeze-etch images on endosymbiotic blue-greens, Bourdu and Lefort (3) have shown that the phycobilisome rows are in a helical pattern following the long axis of the cell. A similar helical orientation is believed to occur in *Synechococcus*. If the row orientation were always unidirectional and perpendicular or horizontal to the cell axis, one would expect to observe only one sectional view of the phycobilisomes in a cross-section of a cell. This is not the

case, however. We have frequently observed more than one sectional view in longitudinal and cross-sections of phycobilisomes in this alga. Short phycobilisome rows are also present and generally are aligned at random with respect to one another. Since short phycobilisome rows have usually been observed in cells where the intrathylakoidal spaces display some swelling, it is difficult to determine whether the short rows are a normal state, or are the results of distortions caused by tortuously oriented lamellae. The three planes of section and their relationship to the underlying membrane are diagrammed in Fig. 2.

DISCUSSION

Phycobilisomes, in the thermophilic blue-green alga *S. lividus*, have a similar structural appearance whether cells are grown in the laboratory (6) or in nature. However, they are more prominent in the organisms collected in their natural habitat. Perhaps, in this study the conditions of the hot springs (e.g., chemical composition of the pool water used during fixation) and the optimum growth of the algae may have contributed to their better preservation.

This alga contains phycocyanin, but no phycocerythrin, and has disc-shaped phycobilisomes as previously found in *Porphyridium aeruginosum* (8, 10).

Particularly striking is the similar spacing of

phycobilisome rows in the red and the blue-green algae. The center-to-center distance of the rows is about 400–500 Å in red algae with disc-type phycobilisomes (containing only phycocyanin), as well as in those with spherical phycobilisomes (containing phycoerythrin and phycocyanin) (8, 12), and also in mesophilic (3, 9) and thermophilic blue-greens (6). Although the significance of this spacing is not yet known, it strongly suggests that the basic structure of the underlying photosynthetic lamella, which is believed to impose the phycobilisome pattern, is similar in both algal groups. Aggregation of phycobiliproteins in vitro occurs in both mesophilic and thermophilic blue-green algae but at different temperatures. Aggregates of high molecular weight have been obtained in vitro with phycocyanin derived from *Phormidium luridum* (1). Phycocyanin from *Synechococcus* has the same properties as phycocyanin of mesophilic blue-greens. The amino acid composition, molecular weight, sedimentation, and antigenic properties are essentially the same. However, the temperature necessary to form large aggregates in *Synechococcus* is 49°C, a temperature considerably higher than that required for optimum aggregation (25°C) in mesophilic phycocyanin.

From the ultrastructural data presented here and from other physicochemical information, speculation on the arrangement of phycobilisomes in *S. lividus* can be made (Fig. 2). It is assumed that these phycobilisomes are composed mainly of aggregated phycocyanin hexamers, since hexamers (125 × 30 Å) are believed to represent the in vivo functional species, as reported for *Plectonema* by Berns and Edwards (2). An average phycobilisome (360 × 320 × 65 Å) of *Synechococcus* could accommodate about 14 hexamers. In order to keep within the phycobilisome dimensions, each hexamer (represented in Fig. 2 by plain circles) is envisioned as aggregated along its broad face with another hexamer to form a dodecamer (1) with a total width of 60 Å and diameter of 125 Å. One must also assume that the dodecamers further aggregate along their narrow edges to form a disc-type phycobilisome. A phycobilisome of this size would have a molecular weight of 2,500,000.

A similar phycobilisome model was proposed by Wildman (13). In our diagram (Fig. 2) the space separating one phycobilisome from another is more extensive and clearly defined. This, however, is not necessarily significant because some varia-

tion in structure (due to species differentiation and also environmental conditions), as well as in interpretation, can be expected. For instance, Bourdu and Lefort (3) suggested, from freeze-etch images, that each phycobilisome has only four subunits in each broad face view (350 Å) versus the seven we suggest. Also, they obtained a phycobilisome thickness of 150 Å in contrast to the 60–70 Å in the present study.

While, at the present time, the data are insufficient for any commitment to a specific model, the physical and structural data thus far obtained in no way contradict available information for the in vivo function of phycocyanin in blue-green algae.

SUMMARY

Phycobilisomes in the thermophilic blue-green alga *Synechococcus lividus*, collected in its natural habitat at 65°–70°C, have been examined. Their structural appearance was found to be similar to that of the same species grown in laboratory cultures. Furthermore, the phycobilisome orientation in parallel rows on the stroma side of the lamellae is the same as in mesophilic blue-green and red algae. A diagram has been presented showing the orientation of the disc-type phycobilisomes of this alga.

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