CYANOPHYCIN GRANULES IN A BLUE-GREEN ALGA *CALOTHRIX MARCHICA* LEMM.

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ABSTRACT

Cyanophycin granules are described from blue-green alga, *Calothrix marchica*. These granules were found in most of the cells, specially in the mature ones and appeared more or less spherical to somewhat elongate; sometimes irregular; 310 to 738 nm in length and 286 to 700 nm in diameter. Their image varied with different fixatives and with the range of temperature of both pre-and post-fixation. Cyanophycin granules fluctuated in numbers in accordance with the cell cycle of light/dark grown cells.

INTRODUCTION

BEFORE the isolation and confirmation of the structure of cyanophycin granules in *Anabaena cylindrica*[1] different workers named these structures differently. The identification of cyanophycin granules was difficult because of their different image with different fixation method and with the variation of temperature of both pre-and post-fixation. Lang *et al.*[1] showed that "Cyanophycin granules"[2] and "Structured granules"[3,4] are one and the same.

These structures were first reported in *Phormidium* sp., *Oscillatoria* sp,, *Anabaena* sp. and *Cylindrospermum* sp.[3] Since then much work[5–9] on the ultrastructure of the blue-green algal cells has been carried out and cyanophycin granules have been described. In the present work, an effort has been made to identify the cyanophycin granules of *Calothrix marchica* on the basis of fitting these published data, particularly with regard to measurements and fixation image. An attempt has also been made to estimate the relative abundance of these granules in the young and mature cells and to relate that with the life-cycle of the alga.

MATERIALS AND METHODS

The species studied was isolated from wet soil, near the margin of a pool in Dacca, Bangladesh. The alga was grown in modified Fogg[10] medium. The culture was maintained in the same medium throughout the experimental studies and was inoculated at 30 ± 1° C in a regime of 6 hours of darkness alternating with 18 hours of light in 3,420 lux.

Fixation was carried out using the following sol - tions:—

(i) 4% gluteraldehyde in 0·1 M cacodylate buffer (pH 7·2) at 4° C;
post-fixation in 1% OsO_4 + 0·1 M cacodylate buffer (pH 7·2) at 4° C,

(ii) Same as above, only the temperature was changed to 20° C,

(iii) Equal parts of 4% gluteraldehyde and 2% aqueous OsO_4 + 0·1 M Cacodylate buffer (pH 7·3) at 4° C,

(iv) 2% aqueous $KMnO_4$ solution. After dehydration in grades of ethanol and cleaning with propylene oxide the specimens were embedded in Taab embedding resin. The LKB 8800A Mark III ultramicrotome was used with glass knives to cut sections. Sections were stained with methanolic uranyl acetate[9]. Zeiss EM9 and siemens Elmiskop IA electron microscopes were employed.

OBSERVATIONS

In section, these granules appear more or less spherical to somewhat elongate; sometimes irregular, often with a protuberance at one end. They vary from 310 to 738 nm in length and from 286 to 700 nm in width. Previous records of their measurements in different blue-green algal cells show that they can be up to 1000 nm in diameter in the vegetative cells.

They occur often near the transverse septa and sometimes near the side walls (Fig. 1), often very close to one or more polyphosphate bodies. They are absent in the heterocysts (Fig. 2).

These granules appear with radiating alternate electron dense and electron transparent striations (Fig. 3), when fixed in 4% gluteraldehyde in cacodylate buffer at 4° C; the same structures appear electron transparent when similarly fixed at 20° C. They appear highly electron-dense, obscuring the structural details, when fixed in mixed gluteraldehyde and OsO_4 in cacodylate buffer at 4° C (Fig. 2). They are dissolved after permanganate fixation leaving an electron transparent space.

Number of Cyanophycin Granules per Cell

Low power electron micrographs containing large numbers of cells of material fixed at different time intervals from subculture, were used for the estimation.

TABLE I

Numbers of cell-sections with different numbers of cyanophycin granules at different time intervals after subculture

Age of culture in hours	Total No. of cell-sections scored	Total No. of cell-sections with no granules	Total No. of cell-sections with 1 granule	Total No. of cell-sections with 2 granules	Total No. of cell-sections with 3 or more granules
12	262	132 50%	90 34%	33 13%	7 3%
18	130	122 94%	6 5%	2 2%	0 0%
24	241	91 38%	71 29%	55 23%	24 10%
40	109	76 70%	22 20%	10 9%	1 1%
66	183	84 46%	64 35%	26 14%	9 5%
90	143	95 66%	29 20%	18 13%	1 1%
116	fl53	129 84%	22 14%	1 1%	1 1%
140	155	96 58%	40 24%	18 11%	12 7%
162	181	133 73%	37 20%	11 7%	0 0%

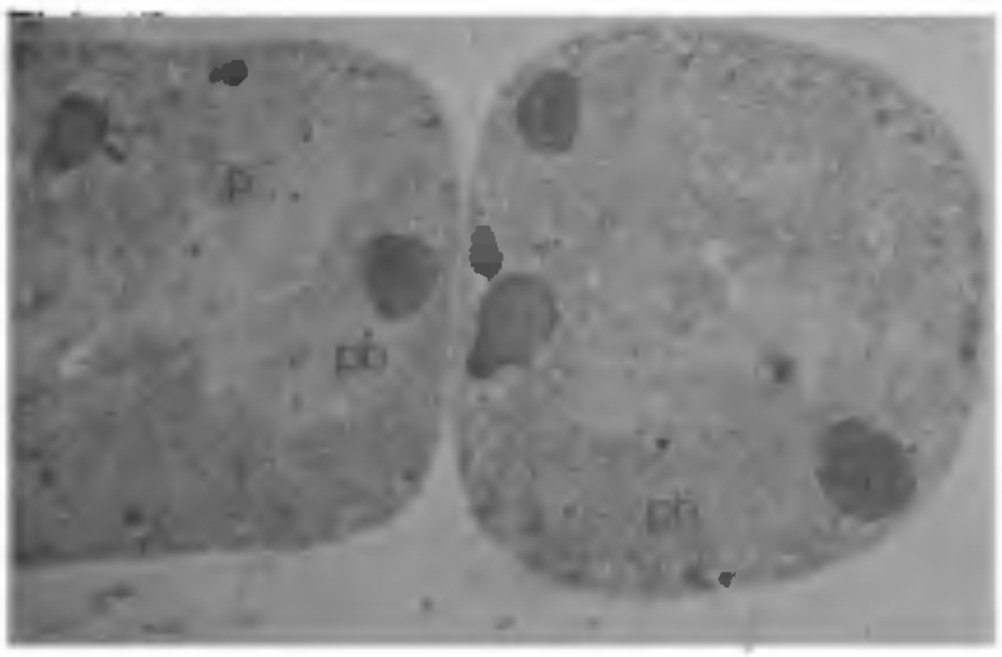

FIG. 1. *Calothrix marchica*, × 22,500. Fixed in 4% gluteraldehyde followed by 1% OsO_4 in cacodylate buffer at 4° C. Electron dense cyanophycin granules (CG) near the transverse sptum and side walls; note presence of polyphosphate bodies (P), polyhedral bodies (Pb) and photosynthetic lamellae (Ph).

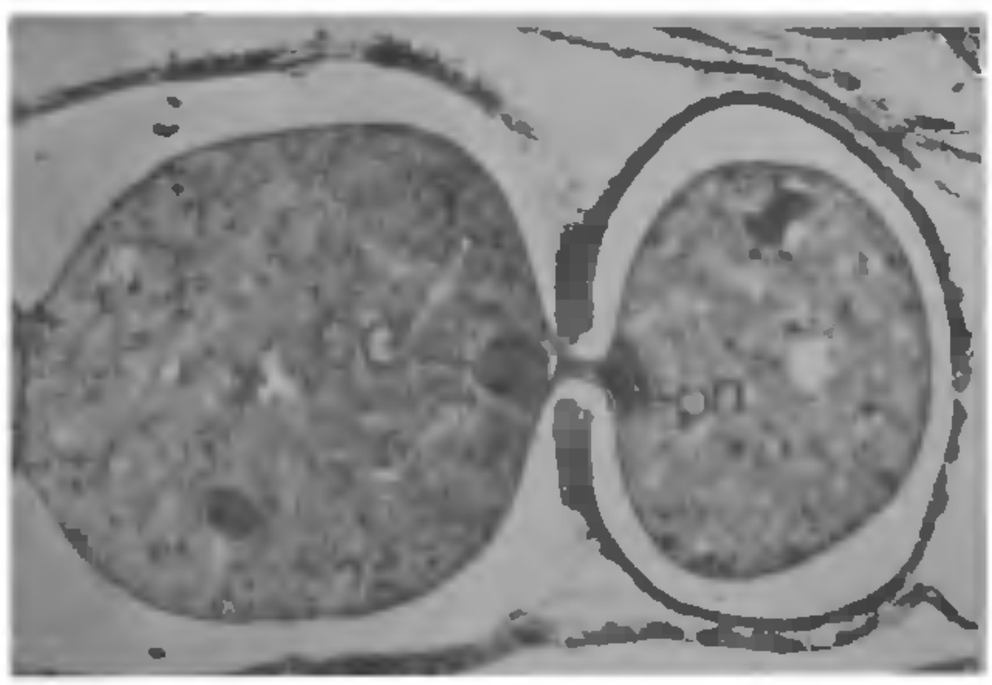

FIG. 2. *Calothrix marchica*, × 9,375. Heterocyst showing electron-dense polar nodule (Pn); cyanophycin granule (CG) absent in the heterocyst but present in the adjacent vegetative cell.

The numbers of electron micrographs at each time interval ranged from four to eleven; the number of cells counted at each time interval ranged from 109 to 262. The total number of cells counted was 1571. The results (Table I) show that there is a regular fluctuation in the numbers of cyanophycin granules per cell at successive time intervals. The numbers recorded are those seen in section; the actual number per cell would undoubtedly be higher. The numbers per cell are at a minimum when the cells are beginning to show active growth, *i.e.*, at 18 hours from subculture and are high at the end of this active period, *i.e.*, at 24 hours. It seems possible that a cycle of alternative building and utilization of cyanophycin granules goes on in resting and dividing cells respectively.

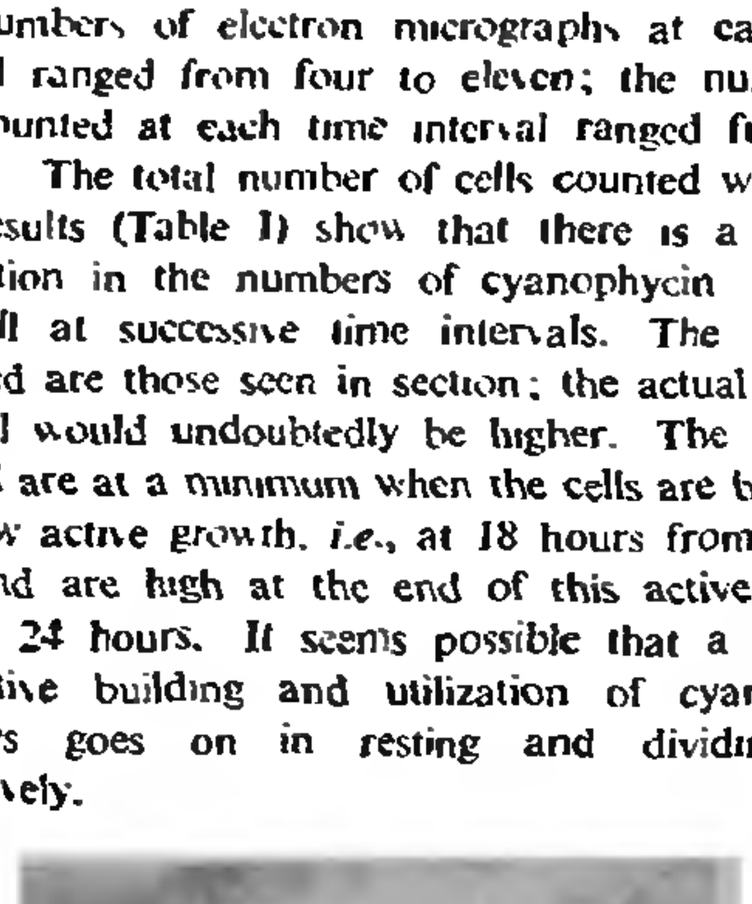

FIG. 3. *Calothrix marchica*, × 120,000. Highly magnified cyanophycin granule showing striations

DISCUSSION

What were probably cyanophycin granules have been described as a proteinaceous food reserve[11,12]. It has been suggested that they are composed of two types of granules—one probably proteinaceous and the other probably lipoidal[5]. That they may be formed from thylakoid membranes and serve as a reserve membrane material is shown in *Nostoc* sp.[13] Recent chemical analysis of granules isolated from *Anabaena cylindrica*[14] did not show the presence of any pigments, lipids or significant quantities of carbohydrates; only two amino acids, arginine and aspartic acid, were detected. It has been suggested by Simon[14] that these granules behave as a storehouse of combined nitrogen, since arginine has four nitrogen atoms per molecule. He has also reported them to be lacking in actively growing cells. In a species of *Cylindrospermum*[2], it has been suggested that cyanophycin granules are utilized during the resting period or very early stages of germination of akinetes.

The present observation of fluctuation in numbers of cyanophycin granules, in the cells of *Calothrix marchica*, which are partly synchronised with cell division, harmonises with the above accounts.

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The First National Symposium on Thermal Analysis will be held under the Joint Auspices of Department of Chemistry, Indian Institute of Technolgoy, Madras and Indian Thermal Analysis Society, during December 21-23, 1978. The last date for receiving abstracts is October 20, 1978. For further details, please contact Dr. M. R. Udupa, Department of Chemistry, I.I.T., Madras 600 036.