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AN ULTRASTRUCTURAL STUDY OF PYRENOIDS FROM *CHLORELLA PYRENOIDOSA*

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SUMMARY

The fine structure of pyrenoids from *Chlorella pyrenoidosa* shows a crystalline matrix with an 8·0-nm periodicity. In certain areas of the pyrenoid 2 sets of parallel lines intersect at approximately 80° to give a criss-crossed appearance to the matrix. The recognition of the crystalline structure depends on the portion of the pyrenoid that is sectioned, as well as the plane of sectioning.

A distinct crystal-like body which is closely associated with the thylakoid lamellae that traverse the pyrenoid matrix is observed in one cell.

Although 2 pairs of thylakoid lamellae run separately through the pyrenoid matrix of a cell, they appear to be continuous with each other outside the pyrenoid.

The fine structure of the pyrenoid, at any given time, seems to be a reflection of its metabolic state. A lack of understanding of the exact function or functions of pyrenoids makes a more rational interpretation of pyrenoid ultrastructure difficult.

INTRODUCTION

Pyrenoids are commonly present in the chloroplasts of many algae and also in the bryophyte *Anthoceros*. The function of these specialized areas in the chloroplasts of these plants is a matter of speculation. Pyrenoids and grana are seldom found in the same chloroplast and thus appear to be mutually exclusive (Clowes & Juniper, 1968).

Most investigations of the fine structure of algae have shown that pyrenoids are composed of a granular, homogeneous matrix around which are found starch deposits (Sager & Palade, 1957; Gibbs, 1962*a*; Bouck, 1965; Chardard, 1965; Evans, 1966; Manton, 1966*a*, *b*; Esser, 1967). Gibbs (1962*b*) and Brown, Arnott, Bisalputra & Hoffmann (1967) have reported a filamentous and fibrillar nature of the pyrenoid matrix in certain green algae. More recently Holdsworth (1968) and Kowallik (1969) have shown a crystalline structure of pyrenoids from the diatom *Achnanthes brevipes* and the marine dinoflagellate *Prorocentrum micans*, respectively. There is no record of the crystalline nature of pyrenoids from any of the other groups of algae except in certain species of brown algae where Evans (1966) recognized a crystalline appearance of parts of the pyrenoids. However, his report does not include any pictures or descriptions of the crystalline structure. As far as we know, the micrographs of *Chlorella* species published to date show only the usual granular structure of the pyrenoids (Albertsson & Leyon, 1954; Murakami, Morimura & Takamiya, 1963; Tamiya, 1963*a*, *b*; Soeder, 1964, 1965; Rodriguez–Lopez, 1965; Staehelin, 1966; Bryan, Zadylak & Ehret, 1967; Wanka & Mulders, 1967; Wanka, 1968; Guerin-Dumartrait,

1968; Budd, Tjostem & Daysen, 1969; Gergis, 1969). It will be of definite interest to report our observations of a crystalline matrix in the pyrenoids of the green alga *Chlorella pyrenoidosa.*

MATERIALS AND METHODS

The stock cultures of *Chlorella pyrenoidosa* (Indiana University culture collection no. 26) were maintained on a light–dark cycle of 14–10 h. Algal cells for electron microscopy were grown in aerated liquid cultures in Bold's basal medium (Parker & Bold, 1961), and samples were taken from a 5-day-old culture by centrifugation. The pellet was fixed in 5% phosphate-buffered glutaraldehyde (pH 7·4) and post-fixed in phosphate-buffered 1% osmium tetroxide (pH 7·4). The cells were washed with phosphate buffer and resuspended in 1% aqueous uranyl acetate (pH 4·0) and then dehydrated in a graded series of ethanols followed by propylene oxide. A tumbling device (Bertagnoli & Nadakavukaren, 1969) was used in all steps of fixation, dehydration and infiltration. The cells were embedded in Epon 812. Sections cut with a diamond knife on a Reichert ultramicrotome were double-stained with uranyl acetate and lead citrate. These were photographed in a Hitachi HU-11A electron microscope operating at 50 kV.

OBSERVATIONS AND DISCUSSION

Usually a single, centrally located pyrenoid is present in the chloroplasts of *C. pyrenoidosa.* Unlike the pyrenoids of diatoms described by Drum & Pankratz (1964) and Holdsworth (1968) there are no specialized membranes found around the pyrenoids of *C. pyrenoidosa.* Starch is commonly present surrounding the pyrenoid matrix (Figs. 1–5). In the vast majority of cells we examined the pyrenoid matrix appeared to be granular and homogeneous.

Occasionally we observed a crystalline structure of the pyrenoid that seems to be present throughout the matrix (Fig. 1). This crystalline structure appears as parallel lines with a centre-to-centre spacing of approximately 8·0 nm. At certain points 2 sets of parallel lines intersect, giving a criss-crossed appearance to some areas of the pyrenoid matrix (Fig. 3). The angle of intersection is approximately 80°. Measurements were made on an enlargement of Fig. 3, and the values stated are averages of the total number of measurements we made in each case.

The two types of lattices in the pyrenoid have been shown to be dependent on the orientation of the crystal with reference to the cutting plane (Holdsworth, 1968). The angle of intersection of the 2 sets of parallel lines and the centre-to-centre spacing of the parallel lines in the crystalline lattice seen in Fig. 3 fall within the range of values reported by Holdsworth (1968).

In one of the cells we found a crystal-like body in the pyrenoid (Fig. 2). This observation is of particular interest as it suggests that the entire pyrenoid matrix need not be crystalline in nature. It is reasonable to assume that there are one or more crystalline areas in the pyrenoid matrix. This may answer the question of why the crystalline nature of a pyrenoid is not observed in all cases. The recognition of the crystalline structure will depend on the portion of the pyrenoid that is sectioned, as well as the plane of sectioning. Holdsworth (1968) has estimated from serial sections that there may be as many as 10–15 crystalline and/or non-crystalline regions composing the 3-dimensional structure of a pyrenoid from *Achnanthes breviceps.*

It is also reasonable to assume that the crystal-like body in the pyrenoid matrix is made up of protein. The presence of protein crystals in plastids of higher plants has been reported by different investigators (Perner, 1963; Manton, 1966*a*; Newcomb, 1967; Shumway, Weier & Stocking, 1967). It has been suggested by Sager & Palade (1957) that the function of pyrenoids in the lower plants is taken over by non-specialized regions of the chloroplasts in higher plants. Assuming the pyrenoids as sites of starch and/or protein storage, this suggestion seems reasonable in light of our knowledge of chloroplast and pyrenoid structure. The significance of the close association of the crystalline body with the hylakoid lamellae which traverse the pyrenoid is not clear.

Frequently a single thylakoid lamella or a pair of closely apposed lamellae traverses the pyrenoids (Fig. 4). It is not uncommon, especially among other algae, to find multiple lamellae in the pyrenoid matrix (Manton, 1966*a*). In one cell we have observed 2 pairs of lamellae in the pyrenoid (Fig. 5). Although they run separately through the pyrenoid matrix, they are actually continuous with one another outside the pyrenoid. The significance, if any, of this is presently not understood. Nevertheless, it suggests the possibility that the multiple lamellae that are occasionally seen in pyrenoids of *C. pyrenoidosa* may actually be sections of a single lamella resulting from the plane of sectioning.

Although much more evidence is needed to propose that at least some portions of all pyrenoids contain a crystalline lattice, recent investigations of pyrenoid ultrastructure in 3 different groups of algae suggest such a possibility. However, the physiological state of the organism at any given time should also be considered as a criterion for the presence or absence of a crystalline lattice in the pyrenoid matrix. There is also evidence from selective staining and fluorescent microscopy that pyrenoids contain protein (Bose, 1941). The crystalline structure of the pyrenoid matrix observed by us and other investigators (Holdsworth, 1968; Kowallik, 1969) further supports this evidence. A lack of understanding of the exact function or functions of pyrenoids makes a more rational interpretation of the pyrenoid ultrastructure difficult at the present time. It has been aptly expressed by Manton (1966*a*) when she said 'it is perhaps a valuable indicator of our ignorance about many matters in which a cytologist must turn to an experimental biochemist for guidance. Granted that photosynthesis is the flywheel of the whole organic world, we need to know not only how a chloroplast is constructed and how it works photosynthetically but also much more than we know at present of the nature of its distribution products, and of the distribution system or systems which convey these from the factory to construction sites elsewhere in the cell.'

Structural studies of pyrenoids from other green algae are already under way in our laboratory. The results of these and other investigations elsewhere should make it possible to have a better understanding of the fine structure of pyrenoids in general.

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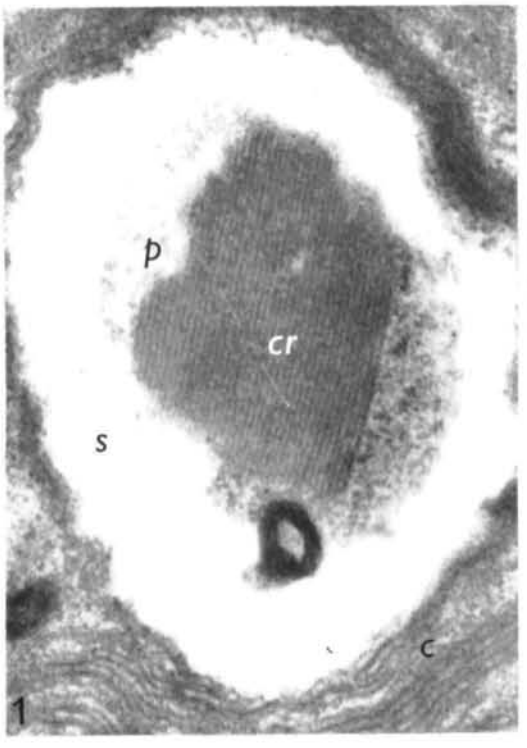
Fig. 1. Section through a chloroplast (*c*) showing crystalline structure (*cr*) of the pyrenoid matrix (*p*); *s* is starch deposit around the pyrenoid matrix. × 120000

Fig. 2. Section through a chloroplast (*c*) showing a distinct crystal-like body (*cr*) in the pyrenoid matrix (*p*). Note the close association of this crystal-like body with the thylakoid lamellae (*t*) that traverse the pyrenoid. (*s*, starch deposit.) × 100000.

Fig. 3. This chloroplast section shows 2 sets of intersecting parallel lines in the crystalline lattice (*cr*) of the pyrenoid matrix (*p*). (*c*, chloroplast; *s*, starch deposit.) × 160000.


p
cr
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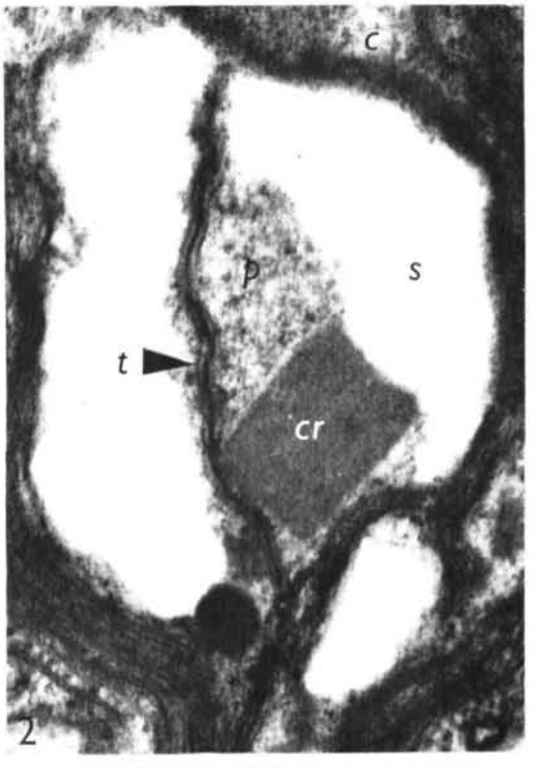
1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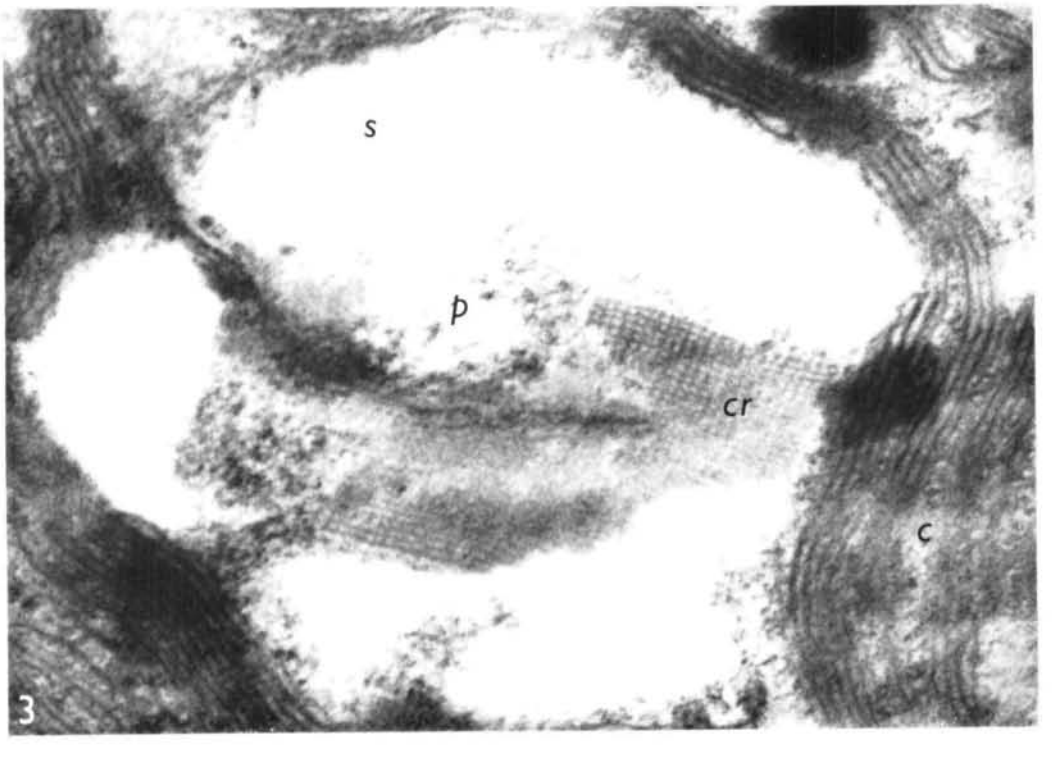
s
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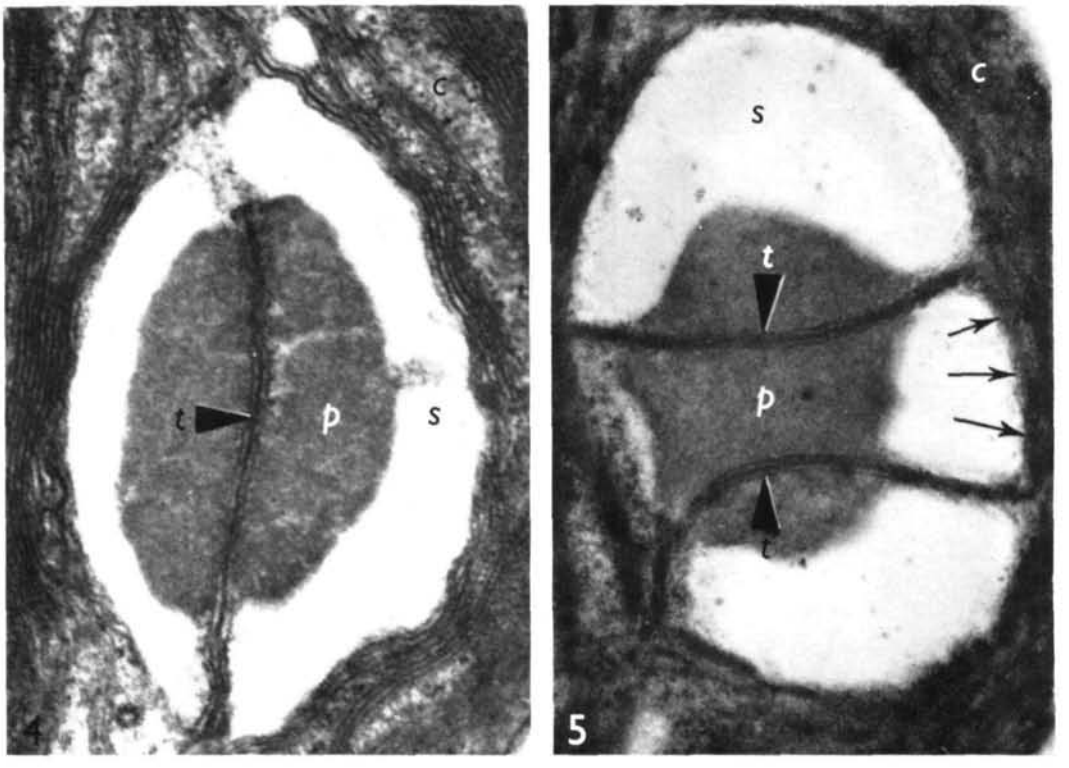

Fig. 4. Section through a chloroplast (*c*). Note the closely apposed pair of thylakoid lamellae (*t*) traversing the pyrenoid matrix (*p*). (*s*, starch deposit.) ×74000.

Fig. 5. Section through a chloroplast (*c*). The 2 pairs of thylakoid lamellae (*t*) that traverse the pyrenoid (*p*) appear to be continuous with each other (arrows). ×80000.