THE PRESENCE OF A CRYSTALLINE MATRIX IN PYRENOIDS OF THE DIATOM, *ACHNANTHES BREVIPES*

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It is generally known that the chloroplasts of most algae contain large bodies called pyrenoids around which form the photosynthetic reserves of the cell (for a general discussion, see reference 1). Most studies on the ultrastructure of algae have shown the pyrenoid to be composed of a finely granular, densely packed, homogeneous material (see references 2–6). Other studies have revealed a fibrillar or filamentous component of the pyrenoid matrix (7–10). There are but few published reports which show the pyrenoid matrix to have an ordered substructure. For example, Evans (11) found that parts of the pyrenoids in some species of brown algae sometimes had a crystalline appearance, but his report includes no pictures or description of the crystal. Joyon and Fott (12) describe the structure of pyrenoids of the green alga *Carteria* as being composed of parallel "plastidious discs" delimiting compartments in each of which are five layers of vesicles 150 A in diameter; although this structure is highly ordered, it was not described as crystalline by the authors.

This paper reports and describes a crystalline matrix in the pyrenoids of the pennate diatom *Achnanthes brevipes.*

MATERIALS AND METHODS

Cultures of *Achnanthes brevipes* Agardh were obtained from the Indiana University Algal Culture Collection and were grown in "diatom seawater" liquid medium (13) in a light–dark cycle, 16 hr of light and 8 hr of dark, at approximately 18°C. The light intensity used was 900 ft-c. Material from 9- and

16-day-old cultures, during the period of vigorous growth, was fixed, in an ice bath, in 4% glutaraldehyde in a 50% solution of the growth medium for 12 hr and then postfixed in 1% osmium tetroxide for 3 hr. The fixatives were buffered at pH 7.3 with 0.1 M sodium cacodylate. Dehydration was effected, in the cold, with a graded series of ethanol followed by propylene oxide. The material was embedded in Epon 812. Silver sections cut on a Porter-Blum ultramicrotome were picked up on 200-mesh uncoated grids, stained with uranyl acetate and lead citrate, and examined in an Hitachi HS 7S electron microscope at an accelerating voltage of 50 kv.

OBSERVATIONS

The pyrenoids of *Achnanthes brevipes* vary in size and shape, but on average they are about 3 μ across. They are never surrounded by starch plates because starch is not a storage product in diatoms (reference 1, page 599).

In section, the crystal-like structure of the pyrenoid matrix appears either as a single array of parallel lines having a center-to-center spacing of 70–80 A (Figs. 2 and 3) or as two intersecting sets of parallel lines giving a criss-crossed appearance (Figs. 1 and 4). In the latter arrangement, the angle of intersection varies with different crystalline regions from 60° to 85°. High magnification of the lattice reveals that the crystal-like structure is composed of arrays of round subunits, each subunit being approximately 50 A in diameter (Figs. 3 and 4). The subunits of the first type of pattern are arranged linearly into rows. The subunits in each row are either packed closely together or linked by short bridges (Fig. 3). Some sections of the criss-crossed or second type of pattern show the subunits to be hexagonally packed. Short bridges link the subunit in the center of each hexagon to the two subunits just above and to each side of it and to the two subunits just below and to each side of it; this gives each hexagon the appearance of having an X across it (Fig. 4). The result is the general appearance of two clearly defined, intersecting sets of parallel lines.

A single pyrenoid may appear to be composed of a single crystalline structure or a number of irregularly shaped crystalline regions distinguishable by type and/or orientation of the lattices (Figs. 1 and 2). Some pyrenoids or regions of pyrenoids have the commonly reported noncrystalline, granular appearance. Although it is difficult to determine the exact number of crystalline and/or noncrystalline regions composing the three dimensional structure of a pyrenoid, it has been estimated from serial sections that in some cases there may be as many as 10–15 regions present. The interface of adjacent crystalline regions may be sharply defined (Fig. 1), or it may be diffuse with the lattices ending, on either side, in a granular zone several hundred angstroms wide running between the regions. At its periphery, the pyrenoid may be bounded by chloroplast lamellae (Figs. 1 and 2) or by a structure similar to the pyrenoid membrane described by Drum and Pankratz (4) (Fig. 2). In either case, the crystal lattices may extend directly to the limiting structure or may end in a granular zone adjacent to it. Regions of the surface of the pyrenoid may be unbounded and may form an interface directly with the chloroplast stroma. In some sections, the chloroplast lamellae appear to end at the surface of the pyrenoid, but no clear physical continuity with the crystal lattice has been seen. Some lamellae are continuous with the double-disc membranes (8, 9) which, when present, run between the different crystalline regions (Fig. 1). The crystal lattice may be directly adjacent to, or separated by a granular zone from, the double-disc membranes. Many sections show relatively small areas in the pyrenoid matrix that contain round, electron-opaque particles (Fig. 2) the diameters of which (120 A) are not significantly different from those of ribosomes found in the chloroplast stroma. Serial sections reveal that these regions are associated with the double-disc membranes and run adjacent to, and to one side of, the membranes as they extend into the pyrenoid (Fig. 2). The "ribosome"-containing regions are continuous with the chloroplast stroma and are not bounded by membranes inside the pyrenoid.

DISCUSSION

The appearance in section of two types of lattices is probably the result of cutting a single type of crystal in different planes rather than an indication of two types of crystals. It is possible to make a model of a crystal which in section shows one or the other of these two patterns depending upon the orientation of the crystal with respect to the cutting plane. In such a model, six bridges link each subunit to six other subunits. Four of the bridges are in the same plane and form two collinear pairs of bridges which intersect at the center of the subunit at an angle of 60°. The

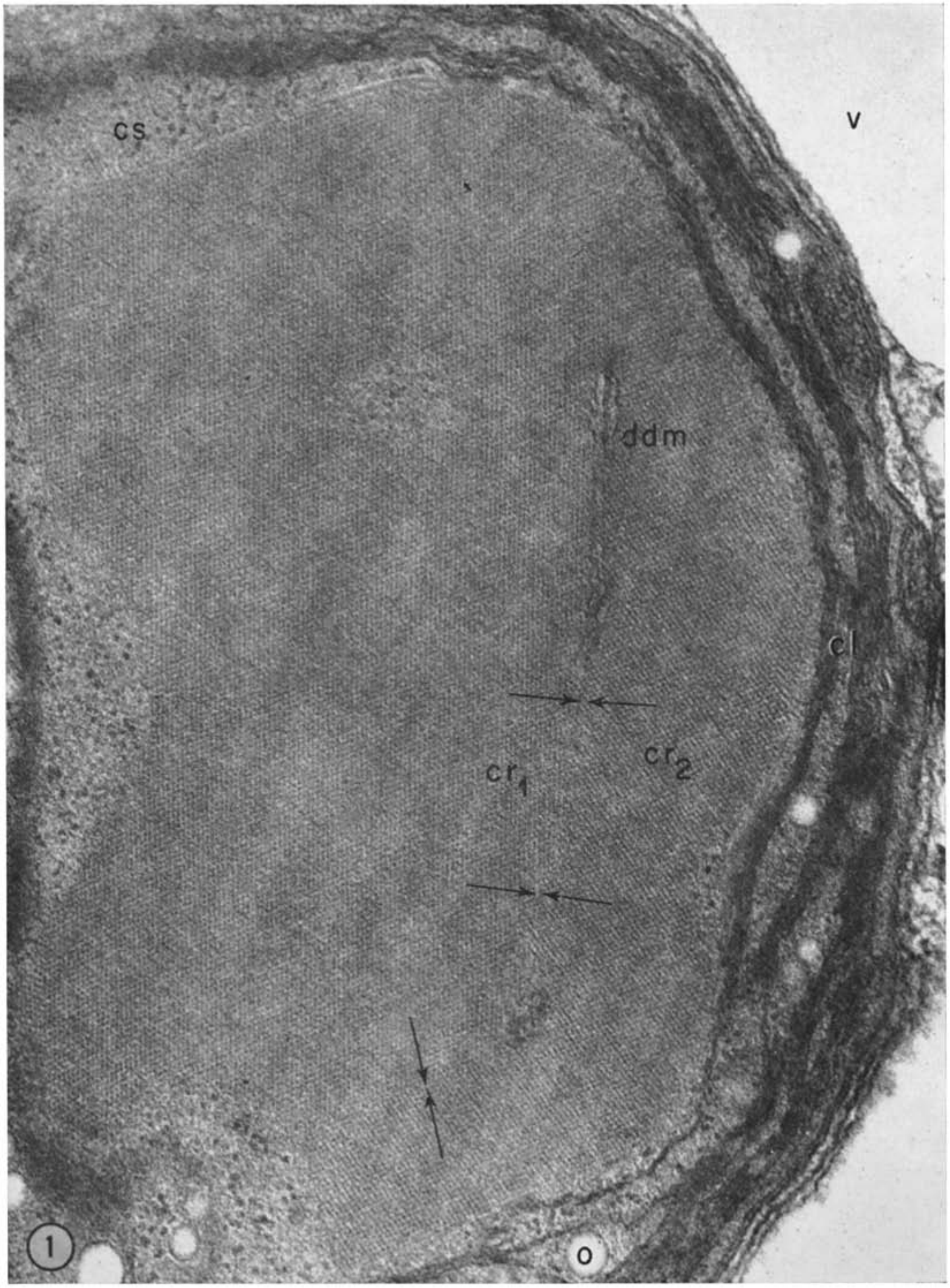

FIGURE 1 Section through pyrenoid showing the criss-crossed lattice. cr_1 and cr_2 label two adjacent crystalline regions whose interface is indicated by the arrows. *cl*, chloroplast lamellae; *cs*, chloroplast stroma; *ddm*, double-disc membranes; *o*, oil droplet; *v*, vacuole. × 109,000.

FIGURE 2 Section through pyrenoid showing adjacent crystalline regions (cr_1 and cr_2) having parallel-line lattices. Arrows about *i* indicate interface of the two regions. *cl*, chloroplast lamellae; *ddm*, double-disc membrane; *o*, oil droplet; *pm*, pyrenoid "membrane;" *r*, ribosomes. × 109,000.

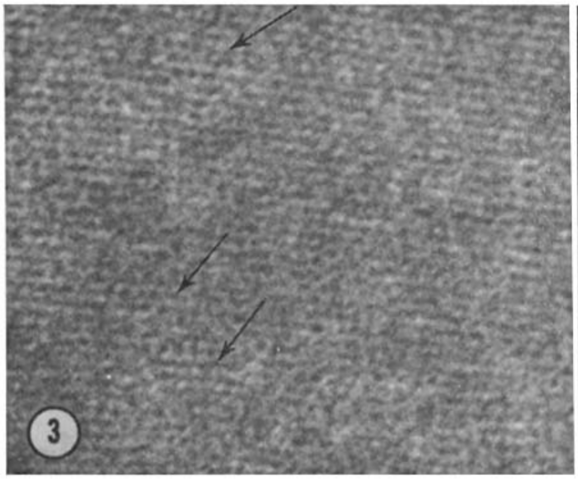
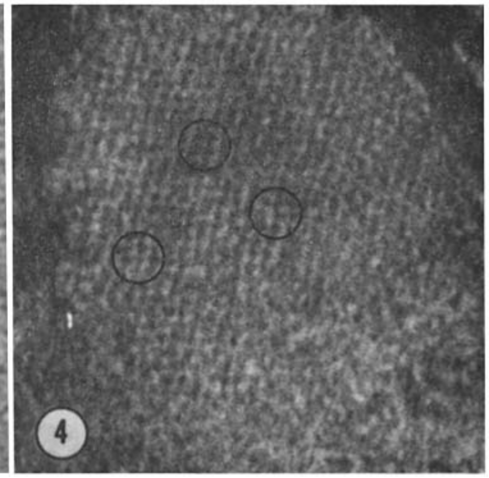

FIGURE 3 High magnification of parallel-line lattice. Arrows point to chains of subunits. × 220,000.

FIGURE 4 High magnification of criss-crossed lattice. Circles enclose hexagonally packed subunits and their linking bridges. × 220,000.

angle of 60° is assumed on the basis of the observed hexagonal packing of the subunits. The other pair of bridges is also collinear and intersects the plane of the first four bridges at an angle of 60°. The orientation of the line formed by the last bridge pair with respect to the two intersecting bridge pairs in the plane is such that the projection of the line onto the plane bisects the 60° angle formed by the two pairs of collinear bridges in that plane (Fig. 5). A surface view of the three-dimensional construct of the crystal model is illustrated in Fig. 6. If the crystal is cut parallel to the plane of face *D* (Fig. 6), one set of parallel lines is seen, each line being formed by a single chain of subunits linked by bridges. If the crystal is cut parallel to the plane of face *A*, *B*, or *C*, two intersecting sets of parallel lines are seen. Variations in the line-to-line spacing and in the angle of intersection, both of which are observed, would be expected, depending upon which of the many possible ways the crystal is cut:

The physical characteristics of the crystal and the possibility that there are pyrenoid-associated ribosomes are consistent with evidence, based on selective staining and fluorescent microscope studies, that pyrenoids are composed primarily of protein (14) and possibly a small amount of RNA as well (reference 2, page 58). The noncrystalline, granular regions, including the zones between crystalline regions and around the periphery,

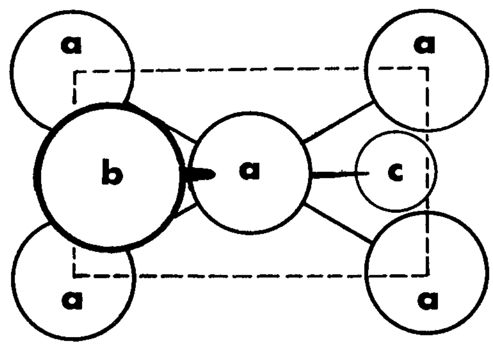

FIGURE 5 Diagram showing three-dimensional relationship of subunits and bridges in the crystal model. Subunits labeled *a* lie in the plane indicated by dashed line; subunit *b* lies above, subunit *c* below, the plane. The line formed by the bridges from *b* and *c* intersect the plane at an angle of 60°.

may be composed of the globular subunits or their precursors in an unorganized state. At present there is no evidence to suggest that the protein of the subunits is synthesized at the sites of the pyrenoid ribosomes, but such an idea is very attractive.

It is interesting to note that the structure of the pyrenoid crystal in *Achnanthes brevipes* is strikingly similar to that of crystals found in chloroplasts of higher plants (e.g. see references 15–17). This similarity may give some support to the idea that

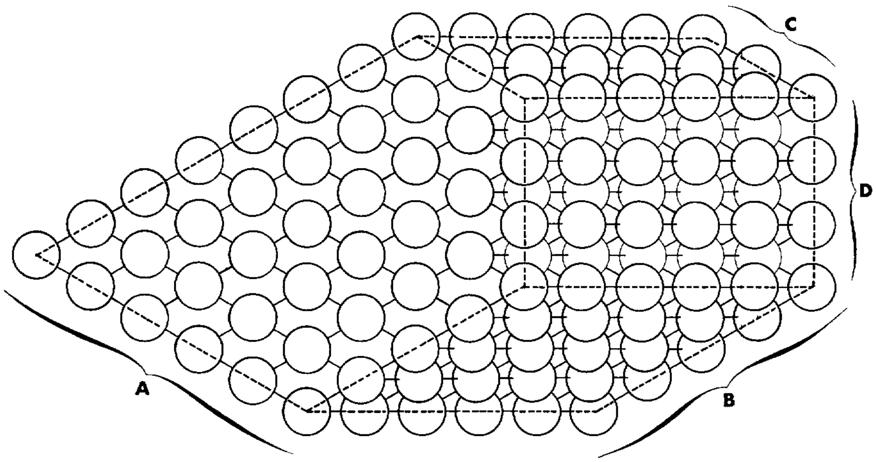

FIGURE 6 Surface arrangement of the subunits in a three-dimensional segment of the crystal model. *A*, *B*, *C*, and *D* represent surface planes of the segment.

the function of pyrenoids in algae is taken over by certain regions of the chloroplast in higher plants (6).

This paper has described the existence of a crystalline pyrenoid in a single algal species. Until further work is done on a broader sampling of algae, the question of whether a pyrenoid-crystal is of general occurrence must remain open.

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