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STRUCTURE OF THE PERIPLAST OF *CRYPTOMONAS OVATA* VAR. *PALUSTRIS*[1,2]

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SUMMARY

The periplast of Cryptomonas ovata *var.* palustris *is composed of polygonal plates which are delineated by shallow ridges. A small ejectosome is located at each corner of the plate area. The plate areas vary in size; they are smallest at the anterior and posterior ends and are largest in the middle of the cell with an average length of 0.5 μ and of width 0.4 μ. In cross section a plate area is composed of 2 distinct layers, an outer plasma membrane layer with a fine particulate appearance, and an inner layer consisting of two sheets. The sheets of the inner layer have a striated lattice pattern with a periodicity of about 20 nm. In negatively stained preparations one lattice appears to underlie another at certain angles. Protease digestion removed polygonal shaped inner layer.*

INTRODUCTION

The surface structure of the periplast of the Cryptophyceae appears to have a highly characteristic and critical taxonomic value in algal classification. So far, the periplast has received little attention in spite of numerous papers published on the ultrastructure of these organisms (*1,2,4–6,8–10*). Because of their size, elucidation of the periplast structure in these algae is difficult by light microscopy. Observations on the periplast of several organisms have indicated a complex architecture (*6*) variously described as to possess occasional striations (*1*), indentations (*2*), hexagonal lattice pattern (*8*), and rectangular plates (*3*).

Cryptomonas ovata var. *palustris* was selected because it has a periplast with a distinct surface structure. The periplast ultrastructure was elucidated through various microscopical techniques and by separating the periplast with sonication. This investigation revealed that *C. ovata* var. *palustris* has a more complicated periplast than *Chroomonas* sp. (*3*). It is composed of 2 distinct layers, the outer layer or plasma membrane and an inner layer containing polygonal plates.

MATERIALS AND METHODS

The culture of *C. ovata* var. *palustris* was originally obtained from the Indiana Culture Collection. Cells were grown on C-base liquid medium essentially described by Hoogenhout & Amesz (*9*). Cultures were constantly illuminated with daylight fluorescent lamps (Westinghouse F 40 D) at 1800μw/cm² and were kept at 20 C.

Cells were fixed in 4% glutaraldehyde with 0.1 M phosphate buffer (pH 7.0) and 0.2 M sucrose. They were postfixed in 1% osmium tetroxide and embedded in Epon as described previously (*6*). Thin sections were stained with lead citrate and examined in a Phillips 300 electron microscope. When cells were treated with trypsin after glutaraldehyde (4%) fixation, they were incubated for 1–4 hr at 37 C with trypsin (50 mg/ml in 0.1 M sodium phosphate buffer pH 7.0). After washing the fixed cells 4 times, control cells were treated identically without the presence of trypsin.

Negative staining was carried out on cells sonicated for

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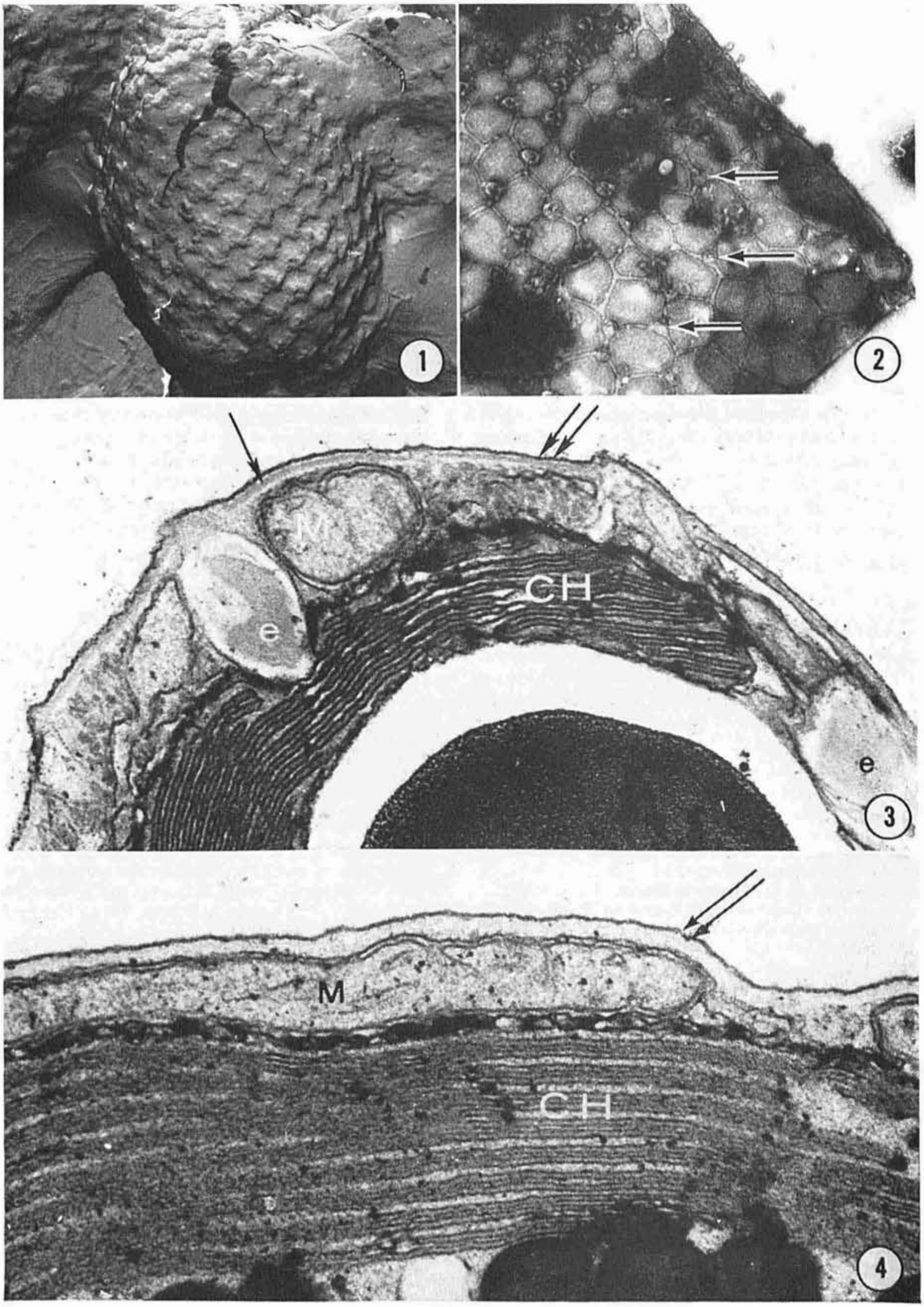
FIG. 1. An electron micrograph of a platinum carbon replica of a whole cell of *C. ovata* var. *palustris*. The surface of the cell shows regular indentations which are composed of platelike areas. × 10,000.

FIG. 2. In this negatively stained (PTA) sonicated periplast preparation, several segregated plates are seen. Upon cell rupture and release of certain tension, the plate area became irregularly shaped. Ejectosome chambers are marked with arrows. × 12,000.

FIG. 3. In thin section the periplast is composed of 2 distinct layers. The outer layer or plasma membrane appears smooth (*double arrows*) and the inner layer is composed of a moderately electron-dense material (*single arrow*). Underneath the periplast a mitochondria (*M*) is present; a portion of the chloroplast (*CH*) and a small ejectosome (*e*) are visible. × 96,000.

FIG. 4. A portion of a cell treated with trypsin demonstrates the disappearance of the inner periplast layer of the polygonal plate. × 96,000.

FIG. 5–7. Prolonged sonication 6–8 sec separated the polygonal plates as shown in this negatively (PTA) stained preparation. FIG. 5. The plates are of various shapes and sizes with a definite lattice pattern. Note the attachment of the outer periplast layer to the plates has a fine granular appearance (*arrows*) and separate ejectosome chambers (*e*). × 35,000. FIG. 6. In this negatively (PTA) stained preparation a single plate is shown. The outer periplast layer is partially torn away, yet in some places still held firmly to the edges of the polygonal plate. Note the fine particulate appearance (*arrows*) of the outer periplast layer and the striations on the polygonal plate. × 96,000. FIG. 7. A single striated polygonal plate is visible. The striated lattice pattern appears about 20 nm in size and 1 set of lattices underlies another set at certain angle. × 96,000.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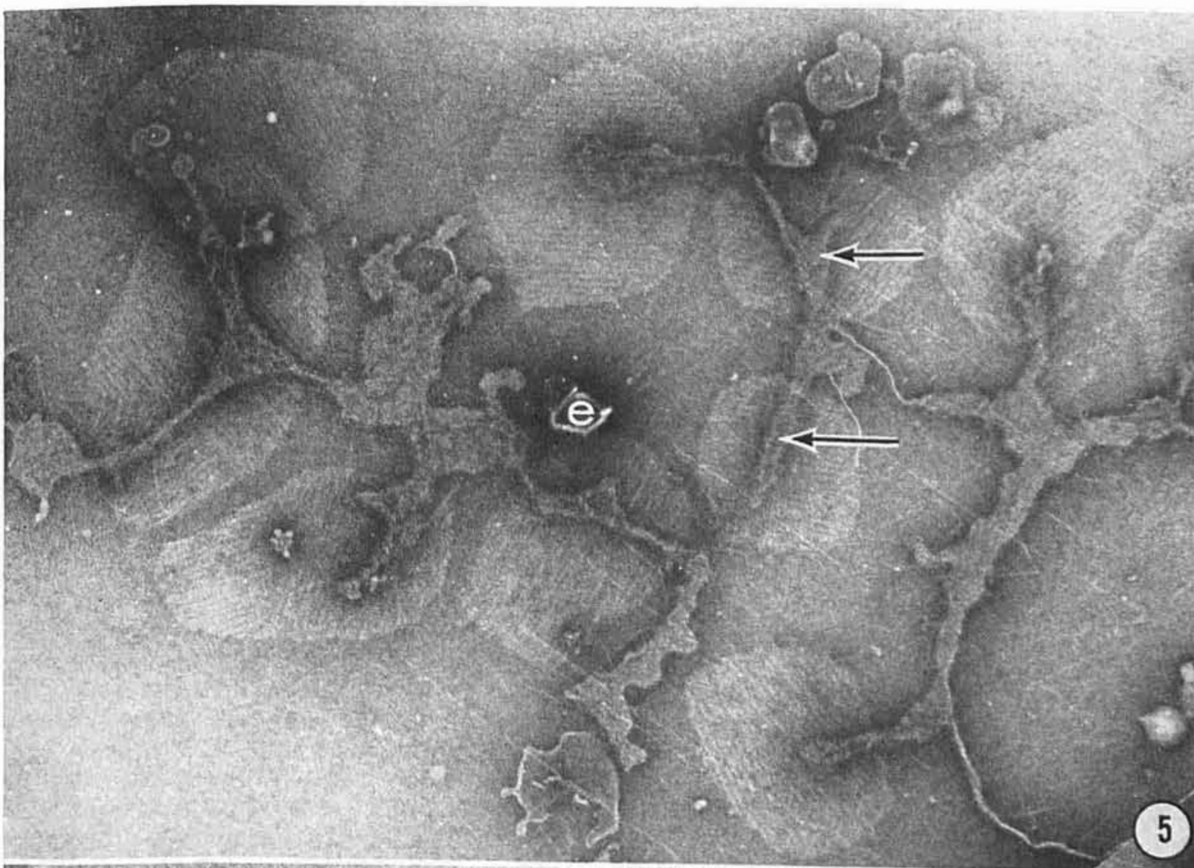
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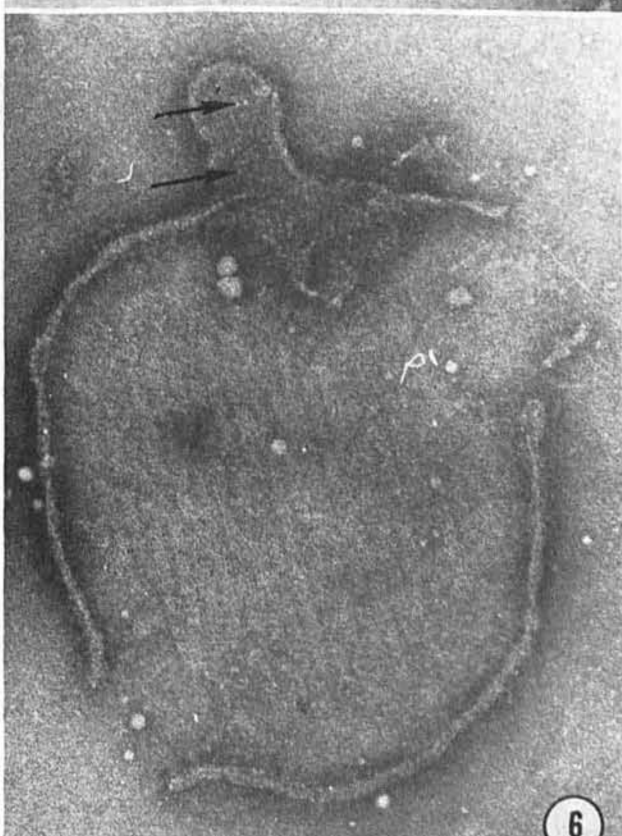
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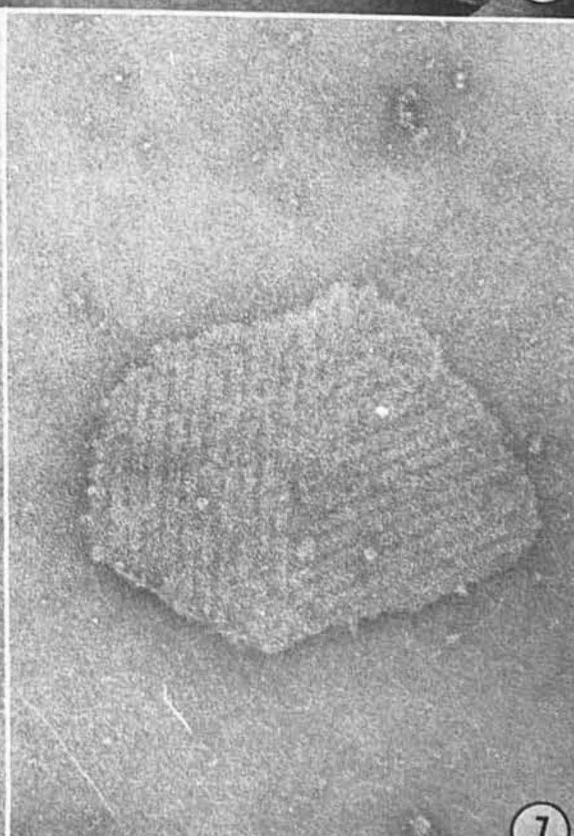
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2–8 sec in distilled water. One drop of cell suspension was mixed with a drop of 2% phosphotungstic acid buffered at pH 7.0 and applied to Formvar-carbon-coated grids.

Replicas were prepared of glutaraldehyde (4%) fixed cells as described by Gantt (5). After several washings in distilled water, cells were applied to freshly cleaved mica, shadowed in a Denton Vacuum Evaporator (Denton Vacuum, Inc., Cherry Hill, New Jersey). Replicas were floated on distilled water and then transferred to chromic acid for 60 min to remove organic material. This was followed by 2 distilled water rinses. The replicas were picked up on Formvar-coated grids and were examined in the electron microscope.

OBSERVATIONS

Cryptomonas ovata var. *palustris* has an ovoid shape (Fig. 1). The basic structure of the periplast consists of an outer single membrane surrounding the cell, beneath which lies an inner layer (Fig. 3). Replicas of an entire cell indicate that the periplast is composed of a regular array of plates in 18–20 longitudinal rows (Fig. 1). These plates are delineated by ridges over the entire cell surface. Each small ejectosome forms a slight bump which has an indented center. The plates vary in size (Fig. 2). The smallest are found in the posterior and anterior ends, while the largest are generally in the middle of the cell. The average length is about 0.5 μ and the average width is 0.4 μ.

Thin sections of the periplast reveal that it is composed of 2 distinct layers (Fig. 3). The plasma membrane constitutes the outer layer and is continuous over the cell surface. This layer appears smooth (Fig. 3 and 4), has a thickness of 100 Å, and has a fine particulate surface appearance seen by negative staining (PTA) (Fig. 6). The inner layer, which has a thickness of 150 Å, is composed of a moderately electron-dense material, and stains less than the outer periplast layer. The inner layer is susceptible to digestion by trypsin as shown in thin sections of control and treated whole cells (Fig. 3 and 4). A firm attraction is present presumably between the inner and outer layer of the periplast, creating a stiffness in the cell contour. The inner layer is segmented and can be detached from the outer layer by sonication (Fig. 5). After this treatment the inner periplast layer separates into polygonal plates following the innate segmentation, and these plates have a definite striated lattice pattern (Fig. 5, 6, 7).

Mild sonication, 1–2 sec, resulted in loss of cell shape due to the occasional splitting of the plasma membrane. It also resulted in the distortion of the regular plate areas and in the release of the ejectosomes (Fig. 2). When sonication was increased to 6–8 sec, a complete separation of the plates occurred (Fig. 5). The outer smooth periplast layer of some separated plates were held still firmly to the edges of the striated polygonal plate (Fig. 6). Additionally, the inner periplast layer was freed from the outer one and can be seen in Fig. 7 as a polygonal plate.

The polygonal plates possess a striated lattice pattern with a periodicity of 20 nm (Fig. 5, 6, 7). In negatively stained preparations, one set of lattices appeared to underlie another set at certain angles. One might speculate that the striated lattice pattern of the polygonal plates provides certain elasticity and tension to the periplast, yet, when the 2 layers, the inner and the outer one, are joined the cell retains its stiffness.

DISCUSSION

This investigation shows that *C. ovata* var. *palustris* has a more complex periplast structure than *Chroomonas* sp., since the latter does not seem to possess the individual polygonal plates (3). Furthermore, the periplast architecture of *C. ovata* var. *palustris* has a multifunctioning uniqueness: it has greater strength than just a continuous membrane, and might provide additional elasticity (the proteinaceous nature of the polygonal plates), rigidity, and flexibility to the structure similar to that provided by a cell wall.

The same type of polygonal plates seen in *C. ovata* var. *palustris* has been observed in replicas of other species, for example, *Cryptomonas* HW 2128, *Rhodomonas lens*, and *Chroomonas salina* (personal observation). On the basis of these observations, one would expect a closer taxonomic relationship of these species than one would expect with *Chroomonas* sp.

At the ultrastructural level, the Cryptophycean algae is a clearly defined group and the periplast architecture and variations have a distinct character. This structure can be used as a taxonomic criterion within the group as well as among other classes of algae.

REFERENCES

1. Butcher, R. W. 1967. An introductory account of the smaller algae of British coastal waters. IV. Cryptophyceae. *Fish. Invest. London Ser. IV*.
2. Dodge, J. D. 1969. The ultrastructure of *Chroomonas mesostigmatica* (Cryptophyceae). *Arch. Mikrobiol.* **69**: 266–80.
3. Gantt, E. 1971. Micromorphology of the periplast of *Chroomonas* sp. (Cryptophyceae). *J. Phycol.* 7:177–84.
4. ———, Edwards, M. R., & Provasoli, L. 1971. Chloroplast structure of the Cryptophyceae: Evidence for phycobiliproteins within intrathylakoidal spaces. *J. Cell Biol.* 48:280–90.
5. Gibbs, S. P. 1962. Nuclear envelope-chloroplast relationship in algae. *J. Cell Biol.* 14:433–44.
6. Hibberd, D. J. 1971. Observations on the ultrastructure of the flagella and periplast in the Cryptophyceae. *Br. Phycol. J.* 6:61–72.
7. Hoogenhout, H. & Amesz, J. 1965. Growth rates of photosynthetic organisms in laboratory cultures. *Arch. Mikrobiol.* 50:10–25.
8. Lucas, I. A. N. 1970. Observations on the fine structure of the Cryptophyceae. I. The genus Cryptomonas. *J. Phycol.* 6:30–8.
9. Mignot, J. P., Joyon, L., & Pringsheim, E. G. 1968. Complements a l'etude cytologique des Cryptomonadines. *Prostistologica* 4:493–506.
10. Schuster, F. L. 1968. The gullet and trichocysts of *Cyathomonas truncata*. *Exptl. Cell Res.* 49:277–84.