The pyrenoid ultrastructure in *Oocystis lacustris* Chodat (Chlorophyta, Trebouxiophyceae)

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Abstract: The fine structure of vegetative cells of *Oocystis lacustris* has been studied with special attention to the ultrastructure of the pyrenoid and its starch sheath. The TEM-investigation showed that the pyrenoid matrix is homogenous, not traversed by thylakoids and the surrounding starch sheath is continuous, horseshoe-shaped or fragmented in 2 starch plates. This starch sheath structure is regarded as a common feature within *Oocystis* and closely related genera *Eremosphaera* and *Neglectella*.

Key words: *Oocystis lacustris*, ultrastructure, pyrenoids, starch sheath

Introduction

Photosynthetic pigments, storage products and structure of plastids are some of the important features in the taxonomy of eukaryotic algae. In many types of algae within the chloroplast occurs a dense proteinaceous body, visible with light microscope and designated as a pyrenoid. The term “pyrenoid” was created by Schmitz (1882) who was the first to associate this structure with an effect on the accumulation of starch grains in the chloroplasts of green algae. Nowadays it is well known that pyrenoids contain the carbon fixing enzyme Rubisco and are commonly associated with formation of storage products (e.g. Graham & Wilcox 2000, Lee 2008). A remarkable number of morphological types of pyrenoids exists (e.g. Dodge 1973, Etzl 1980, Whatley 1993). The absence or presence of pyrenoids in vegetative cells was already used as a taxonomic criterion on the generic level of algae (Starr 1955, Hindak 1977-1990) whereas the morphology of the starch sheath itself, its structure, and location can assist in the identification of green algal species (Brown & McLean 1969, Etzl 1976, Komárek & Fott 1983, Etzl & Gärtner 1988a). The starch sheath of green algal pyrenoids is normally visible with light microscope (Etzl 1980) especially when stained with reagents such as Lugol’s iodine solution. The main structure of the pyrenoid matrix (homogenous, perforated, lamellate or traversed by thylakoids) is also visible in LM with extraordinary optical equipment and when stained with reagents such as azocarmine-G solution (Etzl 1976, 1983, Gärtner 1985). The electron microscopy only cleared characteristic internal details of the pyrenoid matrix (Gibbs 1962, Dodge 1973, Pickett-Heaps 1975, Friedl 1989, Ingolić & Gärtner 1988a). Among the members of the genus *Oocystis* A. Br. the ultrastructure of a pyrenoid with horseshoe-shaped starch sheath was first shown by Schnepp, Koch & Deichgräber (1966, p. 165, fig. 33) in a schematic graph of *Oocystis solitaria* Wittrock f. maior Wille. Later on, Robinson & White (1972, p. 112, fig. 5) presented a pyrenoid in one TEM-micrograph of *Oocystis apiculata* W. West, and recently Soldo et al. (2005, p. 314, fig. 2 A) documented the pyrenoid of *Oocystis nephrocytioides* Fott et Čado in a micrograph of an ultra thin cell section.

In this paper the ultrastructural details of the pyrenoid in cells of *Oocystis lacustris* Chodat are described for first time and comparison with the related genera *Neglectella* Vodeničarov
et Benderliev, Eremosphaera De Bary and Siderocelis Fott is done.

Material and methods

Oocystis lacustris material was obtained from selected phytoplankton samples from Lake Tanganyika dated June–July 2003 when it formed dense populations (Stoyneva et al. 2007) and fixed in acid Lugol’s solution. For detailed description of localities, sampling and methods refer to Stoyneva et al. (2007). For TEM study cells were fixed a) in 3% glutaraldehyd in 0,1 M cacodylate buffer and b) in 1% aqueous O₃O₄ in 0,1 M cacodylatbuffer, dehydrated in acetone and embedded in Spurr’s resine¸ ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963). Electron micrographs were taken with a Tecnai 12 (FEI) microscope equipped with a Gatan ccd camera.

Results

In ultra thin sections most of the vegetative cells of Oocystis lacustris contain one parietal chloroplast filling more than half of the cell size (Figs 1c, 3c). However, sometimes also two chloroplasts, and, occasionally, four or more of them have been observed. Their thylakoids occur in pairs. In each chloroplast one pyrenoid with a homogenous matrix is situated and surrounded by a thick starch sheath (Figs 1p, 2p, 3p). The diameter of the pyrenoid body is between 1 and 1.5 µm; the thickness of the starch sheath is about 0.25 µm. Thylakoids are not traversing the pyrenoid matrix. The starch sheath around the pyrenoid appears like a closed ring (Fig. 2) or as a horseshoe-shaped starch plate (Fig. 3). There can also be a sheath consisting of two starch plates, more or less regular in thickness (Fig. 1 st). Additionally, single lenticular starch grains, which are not in close association with the pyrenoid, are visible inside the chloroplast (Fig. 2s). These stroma starch grains may reach

Fig. 1 Vegetative cell of Oocystis lacustris with 1 chloroplast (c) and a starch sheath (st) consisting of two starch plates around the homogenous matrix of the pyrenoid (p). n = nucleus. Scale bar 1 µm.

Fig. 2 Pyrenoid (p) with homogenous starch sheath and additional stroma starch grains (s) in the chloroplast. n = nucleus. Scale bar 1µm.

Fig. 3 Chloroplast (c) with pyrenoid (p) and homogenous horseshoe-shaped starch sheath. Scale bar 1µm.
considerable dimensions (up to 0.25–0.75 µm). A single nucleus is embedded in the cell lumen (Figs 1n, 2n). The cell wall is multilayered (Figs 1–3). Its appearance in wavy structure, most probably, is a result of fixation and dehydration during preparation.

Discussion

The finding of the multilayered cell wall during this study is in conformity with all previous data, which showed that the cell walls in Oocystaceae BOHLIN are composed of several layers and this diacritic criterion is in accordance with the molecular data (KOMÁREK 1979, HEPPERLE et al. 2000).

The pyrenoids and their starch components are of great value among the main diagnostic features for identifying coccal green algae with light microscope. Their structure can be cleared up by using staining procedures and squashing-method (ETTL & GÄRTNER 1988b, 1995). For further taxonomic investigations of unicellular green algae on species level TEM studies of ultrastructural details of cell components are important. Among them the pyrenoid matrix (homogenous, with invaginations or traversing thylakoids) combined with details of the starch sheath composition are significant. Molecular data below.

In the genus Neglectella VODENIČAROV et BENDERLIEV, generally regarded as close to Oocystis, a pyrenoid with massive, thick continuous and homogenous starch sheath is described (BENDERLIEV 1971, VODENIČAROV & BENDERLIEV 1971). According to the comparative studies of BENDERLIEV (1971) and the text in VODENIČAROV & BENDERLIEV (1971) the pyrenoid of Neglectella is of the same type as the pyrenoid of Ereosphera viridis De BARY. This coincides extensively with the descriptions given by FOTT & KALINA (1962) but is in opposition to SMITH & BOLD (1966, p. 25) where in E. viridis “a number of polygonal starch grains often surround the pyrenoids, especially in aging or nitrogen-deficient cells”. Such discrepancies could be based on different cultivation conditions. Recently in the description of Ereosphera tanganyikae STOYNEVA, GÄRTNER, COQUYT et VYVERMAN (STOYNEVA et al. 2006) some diagnostic features of pyrenoid and starch sheath - visible with light microscope - were included. The starch sheath was shown to contain two plates (STOYNEVA et al. 2006, figs. 37, 39). These results generally coincide with our LM observations on Oocystis lacustris, where continuous starch sheath was detected (STOYNEVA et al. 2007, p. 587) and with our recent TEM
investigations, showing that starch plates do not exceed two in number.

The presence or absence of a pyrenoid is documented for most species of *Oocystis* (e.g. Skuja 1956, Bourrelly 1966, Philipose 1967, Hindák 1977–1990, Komárek 1983, Komárek & Fott 1983, John & Tsarenko 2002), but the organization of the starch sheath was often neglected. A special note about the continuous starch sheath was provided by Fott & Čado (1966) in their LM diagnosis of *Oocystis nephrocytioides*. The TEM photo in Soldo et al. (2005) reveals a pyrenoid with homogenous matrix and bipartite starch sheath. Observations with TEM of *Oocystis apiculata* showed a “bilenticular” pyrenoid type (ChadeFaud 1941, Hori & Ueda 1967) traversed by thylakoids and enclosed by a starch sheath fragmented in two parts (Robinson & White 1972).

Our results on *Oocystis lacustris* coincide partially with the observations on *O. apiculata*. In *O. apiculata*, as well as in *O. lacustris*, the starch envelope around the pyrenoid appears continuous or fragmented in two parts. But *O. apiculata* pyrenoids are traversed by a simple (single) tubular thylakoid system as it is shown by Robinson & White (1972, p.112, fig. 5). This was never observed in cells of *O. lacustris* where the pyrenoid matrix appeared homogenous and was not traversed by thylakoids (Figs 1–3). Therefore it is clear that the type of pyrenoid matrix (homogenous or traversed by thylakoids) still needs further studies and, most probably, is not a diacritic feature in *Oocystis* and in Oocystaceae. However, it seems that continuous or slightly fragmented starch sheath with at most two starch plates is a common diagnostic feature in members of the genus *Oocystis* and its closely related genera *Neglectella* and *Eremosphaera*, but this still has to be verified by further observations with TEM on more species.

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