Changes in Chloroplast Structure during Autospore Formation in *Scenedesmus obtusiusculus*

**Brief Report**

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With 10 Figures

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**Summary**

Changes in chloroplast structure of synchronous cells of *Scenedesmus obtusiusculus* in the late stages of the 15-hours light period are described. The mature cells contain highly lobed chloroplasts packed with starch grains which increase in the chloroplasts with the progress of cell age. The chloroplasts appear to contain closely apposed thylakoid membranes in parallel arrays. Chloroplast division occurs by constriction usually caused by the development of the cell plates of the dividing cell.

1. Introduction

Several studies have reported on physiological and light microscopic aspects of growth and autospore formation in *Scenedesmus obtusiusculus* (Kylin and Das 1967, Das 1968 a and b, Weinberger and Das 1972, Das 1973 a and b). Many reports on the ultrastructure of the cell wall (Bisalputra and Weier 1963, Burczyk et al. 1970, Nilshammar, Waller, and Kylin 1972), and development of pyrenoids (Gibbs 1962, Bisalputra and Weier 1964) in various species of *Scenedesmus* have been published.

It was noticed that marked physiological and biochemical changes occurred as the cell approached maturity (Das 1968 a). The present study examines the changes in chloroplast structure during autospore formation in synchronous culture.

2. Materials and Methods

Cultures of *Scenedesmus obtusiusculus* Chod. (Das 1968 b) were grown and synchronized in the same nutrient medium under the same parameters as described before (Das 1973 a) except that the incubation temperature was at 28 ± 1°C.
Samples were removed for fixation after 5 hours and at 1 hour intervals between 13 and 15 hours in the light period during synchronous growth. The cells were fixed with 2% glutaraldehyde directly in the culture medium for 18 hours in cold (4°C), then washed thoroughly in 0.05 M potassium phosphate buffered at pH 7.3 and post-fixed for 2 hours at room temperature with 1% OsO₄ in the same phosphate buffer. The cells were washed thoroughly in buffer then dehydrated through a graded series of ethanol and propylene oxide, and embedded in Araldite. Sections were cut with a glass knife on a Reichert OMU 2 ultramicrotome and stained with 5% uranyl acetate and lead citrate (REYNOLDS 1963). Sections were mounted on uncoated grids and viewed at 50 kV in an RCA EMU-3H microscope using a 40 µm objective aperture.

3. Results

The juvenile non-dividing cells of *Scenedesmus obtusiusculus* always possess a nucleus with an intact nuclear envelope (see Das 1973 a). Approaching maturity, the mononucleate cells undergo one to three mitoses by cleavage of the parent cell into two to eight autospores (Figs. 1–3, see Das 1968 a). The multiple electron-translucent cell-plates are formed, during mitoses, growing outwards and bisecting the peripherally situated chloroplasts (Fig. 2). The autospores thus formed by the crosswalls remain within the parent cell wall until it is disrupted (Fig. 3). Details of the reproductive stage in this *Scenedesmus* sp. (obtained from the same collection) have recently been published by NILSHAMMAR and WALLES (1974).

Chloroplasts with two or three-layer thylakoids membranes are observed in the early stage (5 hours after the onset of light period) of the cell, and on occasion a number of short thylakoids appear, either two or three together (Figs. 4 and 5). The width of a single thylakoid membrane is about 165 Å. Mature and dividing cells possess highly lobed chloroplasts which are packed

Abbreviations used throughout:

CP = chloroplast; CW = cell wall; D = dictyosomes; ER = endoplasmic reticulum; N = nucleus; S = stroma; Sg = starch; T = thylakoids

Figs. 1–3. Electron micrographs of cell of *Scenedesmus obtusiusculus* during the course of autospore formation

Fig. 1. An initial stage (13 hours) of cleavage (arrow) in the cytoplasm passing through the centre of the cell. Note that the disc-like nuclei are in the course of division on either side of the cleavage fissure. The cell contains large number of starch grains and endoplasmic reticulum. ×21,600

Fig. 2. Later stage (14 hours), during the progress of autospore formation. Note that due to the formation of multiple cell-plates the cytoplasm has been divided into several compartments containing highly lobed chloroplasts packed with starch grains, and endoplasmic reticulum. ×12,000

Fig. 3. The latest stage (15 hours); the autospores have already formed by cross walls and are resting within the parent cell wall. Note that each autospore contains one nucleus, chloroplast with starch grains, and endoplasmic reticulum. ×16,000