

CYTOCHEMICAL DEMONSTRATION OF A SINGLE PEROXISOME IN A FILAMENTOUS GREEN ALGA

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INTRODUCTION

This work was performed in an attempt to establish, in part, the biochemical nature of a microbody-like structure of the filamentous green alga, *Klebsormidium*. There is only one such structure in each cell. It is usually lenticular or discoid and invariably occurs between, and appressed to, the chloroplast and nucleus. The microbody-like organelle divides during mitosis and the division of the chloroplast (5). Another objective was to compare the nature of a microbody of a filamentous alga with the microbodies already described for some unicellular algae and for vascular plants.

Microbodies or microbody-like structures, as determined by structural features, have been observed in the unicellular algae, *Chlorella* (8), *Polytomella* and *Chlorogonium* (9), and *Euglena* (11). These structures have a single limiting membrane and a uniform granular matrix. Microbody enzymes are reported from in vitro studies of *Euglena* (10, 12), and *Polytomella* and *Chlorogonium* (9). However, techniques for the cytochemical localization of catalase were negative on *Euglena*, *Polytomella*, and *Chlorogonium*, and no attempt of cytochemical localization was reported for *Chlorella*.

The explanation for the lack of, or negligible activity of, catalase in *Euglena* has been discussed by Graves et al. (11). In *Euglena*, and other algae grown on acetate or ethanol in the dark, the production of acetyl CoA originates from the 2-carbon substrates rather than from fatty acid metabolism as occurs in β -oxidation in vascular plants, and very little hydrogen peroxide is produced. Also, the glycolate-oxidizing enzyme in several unicellular algae is a dehydrogenase, not an oxidase as in vascular plants (2, 13, 15), hence the absence of a hydrogen peroxide-producing system.

Tolbert (18) suggests that the different pathway of glycolate metabolism is a major difference between vascular plants and unicellular algae. Possible exceptions to Tolbert's suggestion are the reports of glycolate oxidase in a *Chlorella* mutant

(17), and *Scenedesmus* and *Ankistrodesmus* (14). However, the reports on *Chlorella* and *Scenedesmus* seem to be contradictory (14, 15). The recent negative results of attempts of cytochemical localization of catalase support Nelson and Tolbert's (15) report of the absence of glycolate oxidase in many unicellular algae.

MATERIALS AND METHODS

Klebsormidium flaccidum was cultured autotrophically by methods described earlier (4). Filaments 10–14 days old were harvested by filtration during daylight hours when the cells were in interphase. They were treated for 2 hr in phosphate-buffered (pH 6.8) 2% glutaraldehyde and incubated in DAB/H₂O₂ after the general procedure of Novikoff and Goldfischer (16) and Beard and Novikoff (1).

Controls included incubation in media which were without either the H₂O₂ or DAB, or in complete media to which either KCN (0.01 M) or aminotriazole (0.02 M) was added.

After incubation and washing, the material was embedded in 1% agar and postfixed in 2% aqueous OsO₄ for 12 hr. Dehydration was accomplished with 12-hr changes of methoxyethanol, ethanol, and propylene oxide. The material was embedded in Epon. Sections from samples treated with each incubation medium were examined both unstained and after staining with uranyl acetate and lead citrate.

RESULTS

Incubation in DAB/H₂O₂ medium resulted in a very dense deposition of osmium black formed by the reaction of oxidized DAB with OsO₄. The reaction product is definitely localized in the single lenticular microbody of each cell. No electron-opaque deposits were observed in mitochondrial cristae or in any other part of the cell. Fig. 1 illustrates a microbody, its position, and its appearance with conventional glutaraldehyde-OsO₄ fixation when poststained with uranyl acetate and lead citrate. Fig. 2 illustrates the relative density of a microbody after DAB/H₂O₂ incubation but

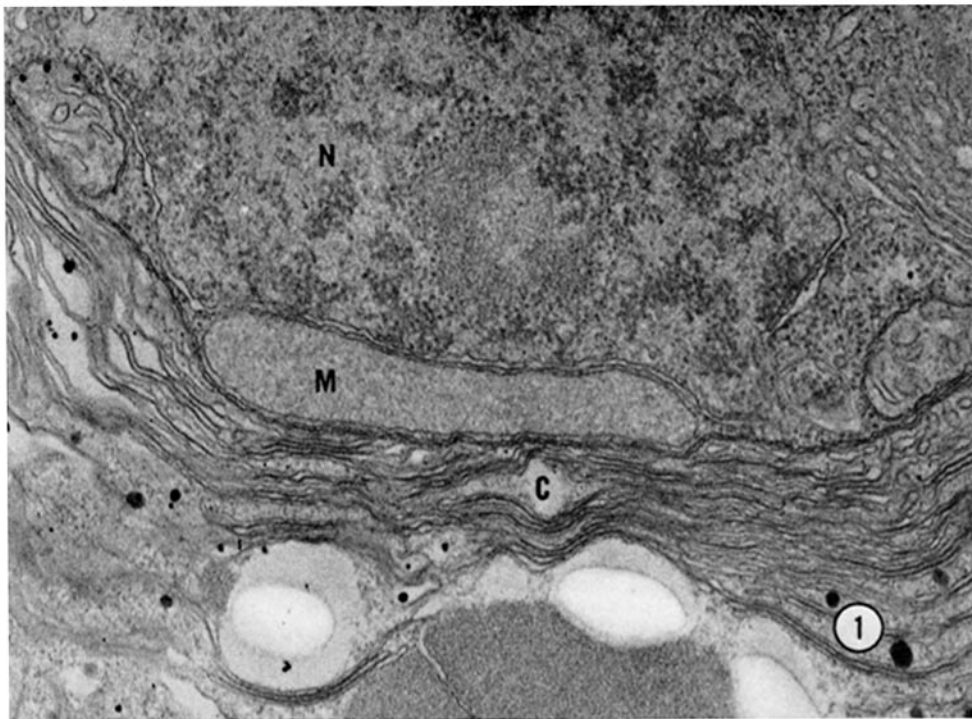


FIGURE 1 The single microbody (*M*) in the usual position between the chloroplast (*C*) and the nucleus (*N*). Cross-section of cell. Glutaraldehyde- OsO_4 , poststained with uranyl acetate and lead citrate. $\times 36,400$.

FIGURE 2 Densely stained microbody (*M*) after incubation in DAB/ H_2O_2 medium. The section was not poststained. Longitudinal section of cell. $\times 43,500$.

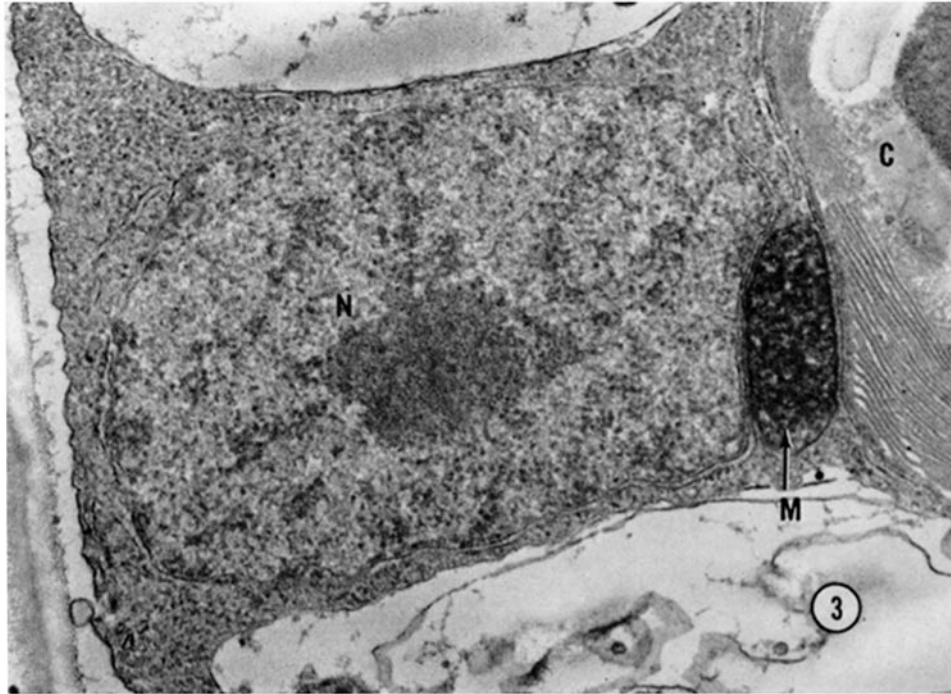


FIGURE 3 Densely stained microbody (*M*) after incubation in DAB/H₂O₂ and poststaining with uranyl acetate and lead citrate. Chloroplast (*C*); Nucleus (*N*) Longitudinal section of cell. $\times 34,400$.

without poststain. After incubation with DAB/H₂O₂ and poststaining, the appearance of the microbody and some other cell structures is as shown in Fig. 3.

Cells incubated in a medium with KCN or aminotriazole were found to be without reaction product. Those incubated in a medium lacking H₂O₂ were also unstained, but those treated with a medium lacking DAB had a slight, but definite, increase in electron opacity of the microbody.

DISCUSSION

The cells of the filamentous green alga *Klebsormidium flaccidum* each contain a single microbody that fits both the biochemical and morphological criteria suggested by others (3, 7, 18). This is the first successful cytochemical localization of peroxidatic activity in the microbody of a green alga. The activity is probably due to catalase (see discussion by Frederick and Newcomb, 6). The slight staining of the microbody of *Klebsormidium* when incubated in a medium lacking DAB is not readily explainable. Frederick and Newcomb (6) reported some reaction product in a medium lack-

ing H₂O₂ and suggested that this was the result of the presence of natural peroxide.

Although glycolate dehydrogenase has been reported in certain unicellular algae (15), the presence of a peroxisome in *K. flaccidum* points to the possibility that glycolate oxidase is present here as is the case in vascular plants. Tolbert (18) has suggested that surveys of the plant kingdom for differences in glycolate metabolism may provide evolutionary and functional clues for peroxisomes. The results of this report support Tolbert's contention since most would agree that the filamentous habit is advanced. Many other filamentous algae should be examined to see whether evolutionary trends are indicated by microbody metabolism. In other filamentous green algae, (*Ulothrix* and *Stigeoclonium*), microbody-like structures have been reported as being smaller, more numerous, and scattered in the cytoplasm (4). Unpublished observations indicate that this is also true of other filamentous genera such as *Draparnaldia*, *Microspora*, *Radrofilum*, *Trichosarcina*, *Schizomeris*, and *Uronema*. In *Coleochaete scutata*, one to several microbody-like structures occupy a posi-

tion between the nucleus and chloroplast similar to the condition described in *K. flaccidum*. These observations suggest that microbodies are of rather general occurrence in the Chlorophyta.

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