

Microbodies from *Spirogyra*

ORGANELLES OF A FILAMENTOUS ALGA SIMILAR TO LEAF PEROXISOMES^{1, 2}

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ABSTRACT

Organelles from *Spirogyra* cells were separated on a linear sucrose gradient. After centrifugation, most of the protein was found in the top fraction. Two minor protein peaks at density (g/cm^3) 1.17 and 1.21 were due to chloroplast particles and mitochondria, respectively. Although there was an extremely low concentration of protein at density 1.25 g/cm^3 , a major part of the activity of glycolate oxidase was found in this region. The enzyme was able to transfer electrons to O_2 and only lost 12% of its activity in the presence of 1 mM cyanide. As documented by electron micrographs, microbodies moved to density 1.25 g/cm^3 during centrifugation. This observation, as well as the fact that high activities of hydroxypyruvate reductase and catalase were also found at the same density, suggest that the microbodies from *Spirogyra* are similar to those in green leaves of higher plants.

Electron microscope studies have firmly established that algae possess microbodies, which are similar in structure to those of higher plants (5, 11, 13). They also contain catalase, a characteristic enzyme of all of these organelles found in different cells (14). There is little information about further constituents of the microbodies in algae.

We have previously reported on the isolation of microbodies from two *Chlamydomonaceae*. After separation of the organelles from these flagellates on sucrose gradients, all of the characteristic peroxisomal enzymes tested were found in the mitochondrial band (15, 16, 18). Microbodies from *Chlorogonium* with a specific density of 1.22 g/cm^3 contained neither enzymes of the glycolate pathway, nor of the glyoxylate cycle, but only catalase and uricase (19). As reported by Gerhardt (4), there are also similar, unspecialized microbodies in *Polytomella*.

Other workers have shown that glycolate can be oxidized in the mitochondria also of two diatoms (12) and of *Euglena* (2). Nevertheless, in *Euglena*, there are microbodies which have the same density and enzymes as the peroxisomes of higher plants (2). However, they contain a glycolate dehydrogenase instead of a glycolate oxidase (1, 10) and are, therefore, different from the microbodies typical of green leaves.

Unlike *Euglena* and several other algae, *Spirogyra* contains a glycolate oxidase (3, 17) which is similar to the higher plant enzyme. The localization of enzymes of the glycolate pathway in *Spirogyra* has been reported in a preliminary article (17) and will be shown more detailed in the present communication.

MATERIALS AND METHODS

Alga and Growth Conditions. *Spirogyra* spec. was obtained from the algae collection of the Institute for Plant Physiology,

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² This communication presents a part of the inaugural dissertation (Habilitationsschrift) of the author.

University of Göttingen. The cells were grown autotrophically at 22 C in the following medium: KNO_3 , 1 mM; $(\text{NH}_4)_2\text{HPO}_4$, 0.08 mM; MgSO_4 , 0.04 mM; CaCl_2 , 0.07 mM; micronutrients as described elsewhere (19), 0.5 ml in 100 ml medium; soil extract, 2 ml in 100 ml medium; vitamin B_{12} , 10 mg/l, 1 drop in 100 ml medium.

The algae were cultivated in 500-ml flasks containing about 100 ml of medium. Light was provided by two fluorescent tubes, at an intensity of about 200 ft-c at the surface of the flasks.

Preparation of Homogenates and Separation of Cell Organelles. The filaments were collected in a sieve with a 1-mm diameter mesh size. The algae were washed several times with water and then once in 20 ml of isolation medium. They were finally transferred to 10 ml of the isolation medium containing the following: Tricine buffer, 0.15 M, pH 7.5; KCl, 10 mM; MgCl_2 , 1 mM; dithiothreitol, 10 mM; EDTA, 1 mM; sucrose, 0.35 M.

Cells were broken by cutting the mass of filaments with scissors for 10 min. The chloroplast bands were also cut into large pieces by this procedure. The crude homogenate was then passed twice through two sintered glass filters with an average pore width of 120 μm and 65 μm , respectively. Most of the chloroplast particles were retained during the second filtration through the 65- μm filter. The crude homogenate, which then had a brown color, was layered on a linear sucrose gradient from 30 to 60% (w/w) sucrose. After centrifugation for 4 hr at 60,000g, the gradient was separated into 1-ml fractions. The sucrose concentration of each fraction was determined with a Zeiss refractometer.

Assays. The protein content of the fractions was measured by the method of Lowry *et al.* (7). To estimate Chl content, 0.05 ml of each fraction was dissolved in 0.95 ml ethanol (96%) and the extinction at 650 nm and 665 nm determined (9).

Enzyme activities were measured in a Unicam SP 800 spectrophotometer. Catalase (EC 1.11.1.6) was assayed according to the method of Lück (8). The activity of Cyt c oxidase (EC 1.9.3.1) was measured by the procedure of Hackett (6), with digitonin being added to each sample (15). The assays for malate dehydrogenase (EC 1.1.1.37) and hydroxypyruvate reductase (EC 1.1.1.26) were as reported previously (16). To determine the activity of glycolate oxidase (EC 1.1.3.1), the following test was used: K-phosphate buffer, pH 8, 0.07 M; dithiothreitol, 1 mM; FMN, 5 μM ; phenylhydrazine-HCl, 2.6 mM; Na-glycolate, 1 mM. The formation of glyoxylate phenylhydrazone was measured at 324 nm.

RESULTS

Two well separated bands were seen within the sucrose gradient after centrifugation of the crude extract from *Spirogyra*. One of these (density 1.17 g/cm^3) contained all of the Chl and, therefore, predominantly chloroplast particles. In the second band around density 1.21 g/cm^3 , about 90% of the Cyt oxidase was found (Fig. 1), indicating that the mitochondria were located mainly in this region. Presumably, most of the mitochon-

dria were not broken during isolation, since a high activity of malate dehydrogenase was also detected at density 1.21 g/cm^3 .

The organelles in the two different bands account for less than 20% of the total protein (Fig. 1). Most of the protein was found in the top fraction of the gradient and, therefore, appeared to be soluble. In the fractions of higher densities, the concentration of protein was very low, and no significant peak or shoulder was detected in the distribution pattern in this region. Nevertheless, about 60% of the total catalase was located in this part of the gradient (Fig. 1), the main activity being found at density 1.25 g/cm^3 , which is typical for leaf peroxisomes (20). In accordance with this observation, electron micrographs of the fraction 1.25 g/cm^3 showed spherical organelles with the characteristic micro-

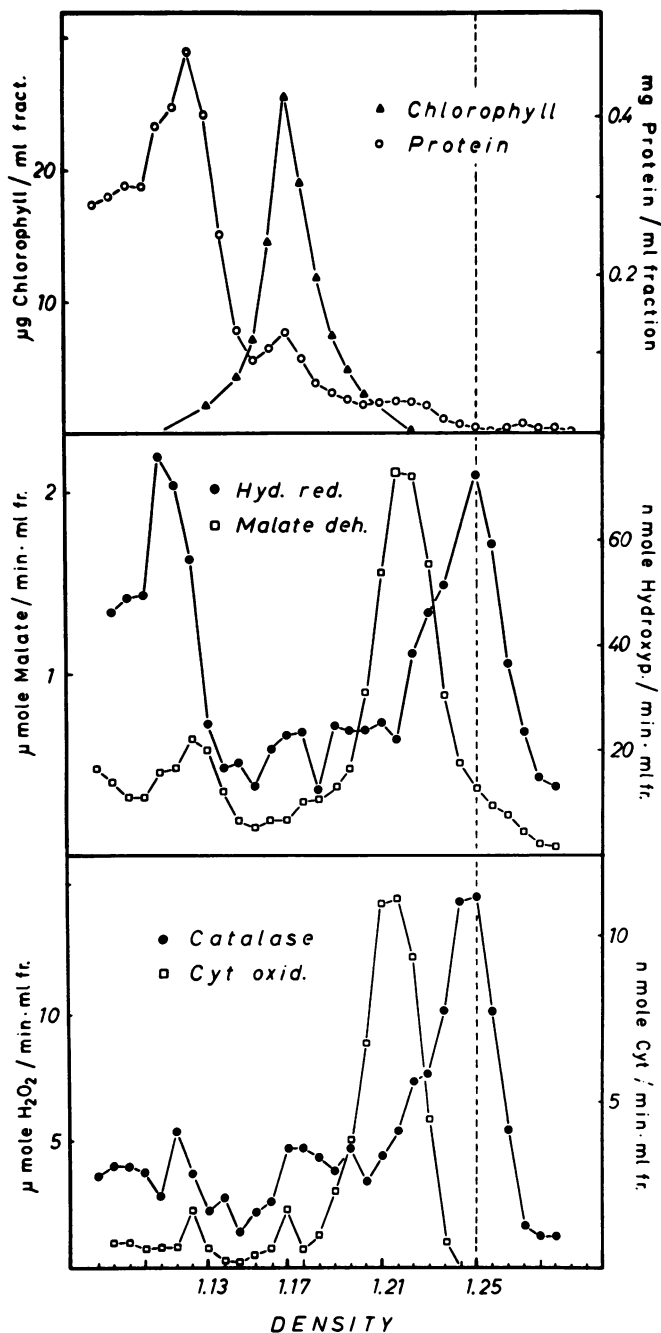


Fig. 1. Separation of cell organelles from *Spirogyra* in a sucrose gradient with linearly increasing density from 1.13 to 1.28 g/cm^3 . Malate deh.: malate dehydrogenase; Hyd. red.: hydroxypyruvate reductase; Cyt oxid.: cytochrome oxidase.

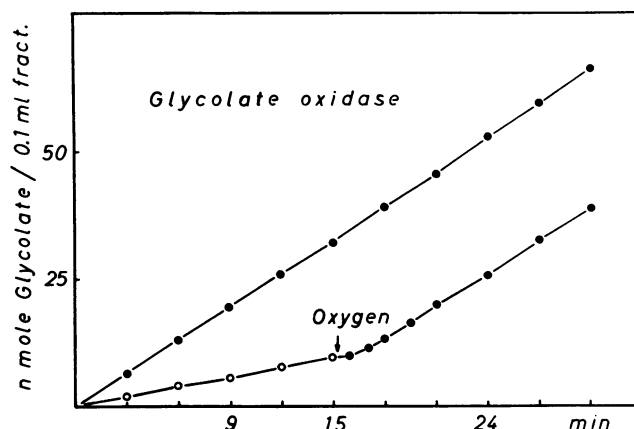


Fig. 2. Determination of the activity of glycolate oxidase from fraction 1.25 g/cm^3 . The glyoxylate phenylhydrazone assay was run in the absence of DCPIP. During the first 15 min, the enzyme activity was monitored in an evacuated cuvette. After aeration of the reaction mixture (arrow), the full activity was reached again. Upper curve: comparison test in the presence of O_2 from air.

body structure, *i.e.* a single limiting membrane and a fine granular matrix. The diameter of these organelles was between 0.4 and $0.8 \mu\text{m}$. Inclusions as in the peroxisomes of higher plants were not seen. In comparison with the number of mitochondria at density 1.21 g/cm^3 , there were considerably fewer microbodies at density 1.25 g/cm^3 , which is consistent with the low protein content of this fraction.

Only 15% of the total catalase was found in the top fraction of the gradient, and about 25% was located in the fractions of lower densities around 1.17 and 1.21 g/cm^3 . The distribution of hydroxypyruvate reductase within the gradient coincided with that of catalase (Fig. 1). However, in the upper fraction, the activity of this peroxisomal enzyme was much higher. Glycolate oxidase, another peroxisomal marker (20), was also detected predominantly at density 1.25 g/cm^3 . The activity of this enzyme could be monitored by measuring glyoxylate formation in the absence of an artificial electron acceptor (Fig. 2). It may, therefore, be capable of transferring electrons to molecular O_2 , like the enzyme of higher plants. In accordance with this observation, the activity of the glycolate oxidase was diminished to a large extent, when tested in an evacuated cuvette without DCPIP.³ However, after aeration of the reaction mixture, the activity was fully restored to that found under aerobic conditions (Fig. 2). The activity of the glycolate oxidase was not affected by 0.1 mM cyanide, and was reduced only marginally (5–12%) at 1 mM cyanide concentration. However, glycolate oxidase was completely inhibited in the presence of 10 mM cyanide.

DISCUSSION

The distribution pattern of Chl and of the different enzymes indicates that separation of organelles from *Spirogyra* was achieved in the sucrose gradient. According to the peak of Cyt oxidase, mitochondria predominantly moved to density 1.21 g/cm^3 , whereas chloroplast particles migrated to a specific density of 1.17 g/cm^3 during centrifugation. About 20% of the total protein was found within the gradient, and as shown by the distribution pattern, this was due to chloroplast particles and mitochondria. The protein concentration was very low in the fractions or higher densities. Nevertheless, the relatively high activity of catalase at density 1.25 g/cm^3 indicates that microbodies moved to this region during gradient centrifugation. In accordance with this observation, electron micrographs of the

³ Abbreviation: DCPIP: 2,6-dichlorophenolindophenol.

fraction 1.25 g/cm³ showed spherical organelles with a diameter of 0.5 to 0.8 μm and with a typical microbody structure. Since only 15% of the catalase was found in the top fraction, it can be concluded that most of the microbodies remained undamaged when the cells were broken. However, only 60% of the organelles reached their equilibrium density, while 25% were apparently trapped in the chloroplast and mitochondria bands, respectively, as indicated by catalase activities in the corresponding regions of the gradient.

In addition to catalase, hydroxypyruvate reductase and glycolate oxidase (17) were also found to be constituents of the microbodies from *Spirogyra*. These organelles are, therefore, of the peroxisomal type found in the green leaves of higher plants (20). Like the leaf peroxisomes, they contain a glycolate oxidase with the ability to transfer electrons in DCPIP (17) as well as to O₂. In accordance with this finding, the enzyme activity was only little affected by 1 mM KCN, whereas the glycolate dehydrogenase of several other algae is almost completely inhibited under the same conditions (10). However, at 10 mM KCN, no activity of glycolate oxidase from *Spirogyra* could be detected at all, whereas the enzyme of higher plants even then does not lose any activity (10).

Almost no activity of Cyt oxidase was found at density 1.25 g/cm³, which shows that the contamination of mitochondria in the microbody fraction was relatively low. Nevertheless, the activity of malate dehydrogenase, which was found to be mainly in the mitochondria of *Spirogyra*, still could be detected at density 1.25 g/cm³ and, therefore, may be also a constituent of the microbodies. The fact that there are many more mitochondria than microbodies in *Spirogyra* may explain the lack of a distinctive shoulder of malate dehydrogenase in the mitochondria band.

According to the distribution pattern, it seems unlikely that peroxisomal enzymes may be located in the mitochondria of *Spirogyra*, as has been suggested for some other algae, but rather, exclusively in microbodies, which so far as is known, are similar to peroxisomes from leaves of higher plants.

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