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Enzymes of β-Oxidation in Different Types of Algal Microbodies

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ABSTRACT

The algae *Mougeotia* and *Eremosphaera* were used for isolation of microbodies with the characteristics of leaf peroxisomes and unspecialized peroxisomes, respectively. In both types of organelles, the following enzymes of the β-oxidation pathway were determined: acyl-CoA oxidoreductase, enoyl-CoA hydratase, and 3-hydroxyacyl-CoA dehydrogenase. There are indications that the peroxisomal oxidoreductase of both algae is a H_2O_2-forming oxidase rather than a dehydrogenase.

The enzymes enoyl-CoA hydratase and acyl-CoA oxidoreductase are located also in the mitochondria from *Eremosphaera* but not from *Mougeotia*. The mitochondrial acyl-CoA oxidizing enzyme was found to be a dehydrogenase. The specific activities of acyl-CoA oxidase and enoyl-CoA hydratase are lower than in spinach leaf peroxisomes. However, the activity of 3-hydroxyacyl-CoA dehydrogenase in the peroxisomes of both algae is almost 2-fold higher. The capability for degradation of fatty acids is a common feature of all different types of peroxisomes from algae.

Microbodies have been demonstrated by electron microscopy to be present in algae of almost every taxonomic division (14). In many of these organelles, catalase could be identified by the cytochemical test with diaminobenzidine. However, further information on enzymic constituents is sparse.

Only from a few algae have microbodies been isolated for biochemical characterization. Summarizing the different results, it seems that there are three groups of algae, one with organelles similar to leaf peroxisomes, another one with glyoxysomes, and a third one with so-called unspecialized peroxisomes containing mainly catalase and uricase (18).

Previously microbodies have been isolated from the filamentous alga *Mougeotia* as well as from the unicellular organism *Eremosphaera* and constituent enzymes were determined. From the data obtained, it became apparent that the organelles from *Mougeotia* are highly similar to leaf peroxisomes and therefore are predominantly involved in glycolate metabolism (18, 20, 22). On the other hand, in *Eremosphaera*, enzymes of glycolate metabolism are located mainly in the mitochondria. In the peroxisomes isolated from this alga, only catalase and uricase could be demonstrated (18, 21). Thus, the organelles from *Eremosphaera* appear to be similar to those from *Chlorogonium* and some other unicellular algae which have been regarded as unspecialized peroxisomes (4, 15, 19).

As has been reported recently for higher plants, leaf and unspecialized peroxisomes both contain enzymes of the β-oxidation pathway (6, 7) which so far were found in glyoxysomes only (3, 8, 11).

As for the algae, up to the present, *Euglena* was the only organism which has been demonstrated to possess peroxisomes with enzymes of the β-oxidation pathway. These organelles also contained enzymes of the glyoxylic acid cycle and therefore were of the glyoxysomal type (8).

The following data show that the algal peroxisomes of the leaf and unspecialized type isolated from *Mougeotia* and *Eremosphaera*, respectively, contain enzymes of the β-oxidation pathway, too.

MATERIALS AND METHODS

Algal Material and Growth Conditions. *Mougeotia sp.*, strain 168.80, and *Eremosphaera viridis*, strain 7180 were obtained from the algae collection of the Institute for Plant Physiology, University of Göttingen. The algae were grown autotrophically at 25°C in continuous light of 3,000 lux and in a medium as previously used (17). The cultures were aerated with air plus 2% (v/v) CO_2.

Preparation of Cell Homogenates and Separation of Organelles. All steps for preparation of homogenates and separation of organelles in a linear gradient from 30 to 60% (w/w) sucrose have already been described (20).

Assays. Acyl-CoA oxidoreductase was assayed by measuring the rate of palmitoyl-CoA-dependent O_2 uptake or DCPIP[2] reduction, respectively.

For determination of the acyl-CoA oxidase activity, a Clark-type electrode was used. The test medium (2 ml; air saturated at 25°C) contained 500 μl of enzyme fraction, 50 mM K-phosphate, pH 7.5, 25 μM FAD, and 50 μM palmitoyl-CoA (Sigma). In accordance with Gerhardt (6), the O_2 consumption could be increased by a factor of 1.7 when 2 mM KCN was added to the test medium. Therefore, KCN was used for all assays.

Acyl-CoA dehydrogenase was assayed with DCPIP as electron acceptor according to Hryb and Hogg (9). The test medium (1-ml final volume) contained 100 μl of enzyme fraction, 100 mM K-phosphate, pH 7, 0.07% (v/v) Triton X-100, 66 μM DCPIP, 1 mM phenazine methosulfate, 2 mM KCN, 4 mg/ml BSA, and 50 μM palmitoyl-CoA (Sigma).

The activity of enoyl-CoA hydratase was determined by measuring the reduction of NAD in the presence of 3-hydroxyacyl-CoA dehydrogenase (13). The test medium (1 ml) contained: 175 mM diethylamine buffer, pH 9.5; 2 mM EDTA, 300 μM NAD; 2 units of 3-hydroxyacyl-CoA dehydrogenase (Sigma); 100 μM crotonyl-CoA (Sigma) and 100 μl enzyme fraction.

The activity of 3-hydroxyacyl-CoA dehydrogenase was determined by measuring the oxidation of NADH (13). The test medium (1 ml) contained: 5 mM K-phosphate, pH 7.5, 2 mM EDTA, 300 μM NADH, 1 mg BSA; 130 μM acetoacetyl-CoA (Sigma), and 100 μl enzyme fraction.

All other enzymes were assayed as described previously: Cyt *c*

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[2] Abbreviation: DCPIP, 2,6-dichlorophenolindophenol.

oxidase, fumarase, and catalase (16); uricase (12, 19). Protein was determined by the method of Lowry *et al.* (10).

RESULTS

Organelles from *Mougeotia* were separated on a linear sucrose gradient. As indicated by the distribution of catalase, peroxisomes moved to density 1.24 $g \cdot cm^{-3}$, whereas mitochondria equilibrated at density 1.20 as demonstrated by the peak of Cyt oxidase (Fig. 1).

Since 75% of the catalase and 55% of the fumarase (total algal cell activities) were found within the gradient the percentage of intact peroxisomes and mitochondria is expected to be relatively high. According to the distribution of enzymes, the peroxisomal fraction at density 1.24 $g \cdot cm^{-3}$ is almost free of mitochondria. However, in the mitochondrial fraction at density 1.20 $g \cdot cm^{-3}$, some catalase activity was detected which obviously represents some trapped, intact peroxisomes.

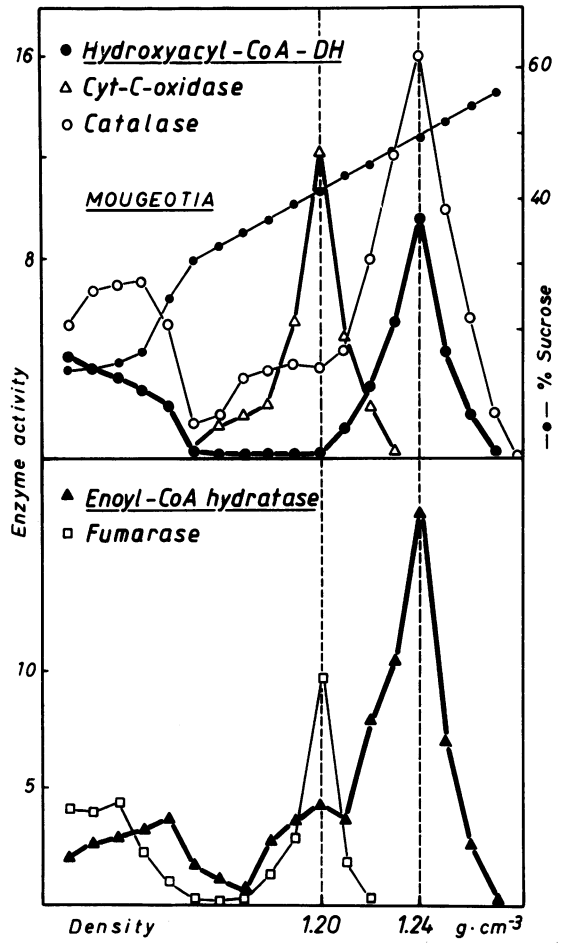

FIG. 1. Distribution of enzymes in a linear sucrose gradient after separation of organelles from *Mougeotia*. Units per ml of fraction per min: 3-hydroxyacyl-CoA dehydrogenase, nmol substrate; Cyt *c* oxidase, nmol substrate (ordinate values × 4 = actual values); catalase, μmol substrate; enoyl-CoA hydratase, nmol substrate; fumarase, nmol substrate.

Three enzymes of the β-oxidation pathway were determined in the peroxisomes of *Mougeotia* (Fig. 1, Table I). Whereas acyl-CoA oxidoreductase and 3-hydroxyacyl-CoA dehydrogenase are located exclusively in the peroxisomes, some activity of enoyl-CoA hydratase was found also in the mitochondrial fraction.

The activity of the acyl-CoA oxidoreductase in the test with DCPIP was relatively high, but the capability also to use O_2 as an electron acceptor may indicate that the enzyme in *Mougeotia* is an oxidase rather than a dehydrogenase (Table I).

After separation of organelles from *Eremosphaera*, mitochondrial and peroxisomal marker enzymes were detected at the lower densities 1.19 and 1.23 $g \cdot cm^{-3}$, respectively (Fig. 2). From the distribution pattern of Cyt *c* oxidase, fumarase, and uricase, we conclude that (as in *Mougeotia* gradients) more than 50% of the mitochondria and peroxisomes from the crude homogenate, which moved to their specific densities during gradient centrifugation, are intact.

Some activity of catalase in fraction 1.19 may be due to trapped peroxisomes in the mitochondria fraction. On the other hand, the percentage of mitochondria in the peroxisomal fraction is very low, as indicated by the activities of fumarase and Cyt *c* oxidase at density 1.23 $g \cdot cm^{-3}$.

In the *Eremosphaera* gradient, all three enzymes of β-oxidation tested were detected not only in the peroxisomes but also in the mitochondria fraction (Fig. 2, Table I). However, the activities of 3-hydroxyacyl-CoA dehydrogenase and O_2-dependent acyl-CoA oxidoreductase at density 1.19 $g \cdot cm^{-3}$ were very low and probably due to trapped peroxisomes.

More than half the total activities of 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase from the crude homogenates of both algae were found to be bound to particles which moved into the gradients during centrifugation (Figs. 1 and 2).

DISCUSSION

From the results presented in this paper, it becomes evident that the leaf and unspecialized types of peroxisomes from *Mougeotia* and *Eremosphaera*, respectively, both contain at least three enzymes of the β-oxidation pathway.

In *Mougeotia*, acyl-CoA oxidoreductase and 3-hydroxyacyl-CoA dehydrogenase are located exclusively in the peroxisomes, whereas some activity of enoyl-CoA hydratase was found also in the mitochondria fraction. However, some peroxisomes may be trapped in fraction 1.20; therefore, it is questionable whether enoyl-CoA hydratase really is a constituent of the mitochondria from *Mougeotia*.

Table I. *Specific Activities of Three β-Oxidation Enzymes in the Mitochondrial and Peroxisomal Fractions of Mougeotia and Eremosphaera Gradients*

The values were determined from the peak tubes.

	nmoles of substrate × min^{-1} × mg $protein^{-1}$			
	Mougeotia		*Eremosphaera*	
	Mitochondria	Peroxisomes	Mitochondria	Peroxisomes
Acyl-CoA oxidoreductase				
O_2-dependent activity	0	8.6	1.2	13.1
DCPIP-dependent activity	0	56.8	8.6	3.2
Enoyl-CoA hydratase	6	107	271	232
3-Hydroxyacyl-CoA dehydrogenase	0	66.2	13.5	65.2

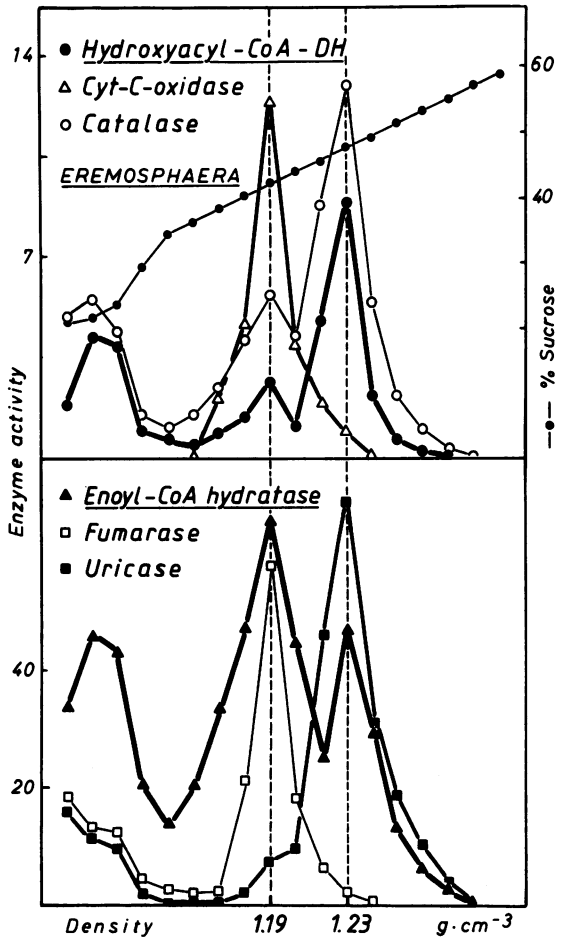

FIG. 2. Distribution of enzymes in a linear sucrose gradient after separation of organelles from *Eremosphaera*. Units per ml of fraction per min: 3-hydroxyacyl-CoA dehydrogenase, nmol substrate; Cyt *c* oxidase, nmol substrate; catalase, μmol substrate; enoyl-CoA hydratase, nmol substrate; fumarase, nmol substrate (ordinate values × 0.2 = actual values); uricase, nmol substrate, (ordinate values × 0.05 = actual values).

The acyl-CoA oxidoreductase in the peroxisomes of *Mougeotia* could be assayed with either DCPIP or O_2 as the electron acceptor. Furthermore, the activity was increased when KCN was added to the test medium. Since catalase, also present in the microbody fraction, is inhibited by cyanide, the increase of the enzyme activity indicates that H_2O_2 is formed during oxidation of acyl-CoA. Therefore, the enzyme apparently is an oxidase as in higher plants which may also show a dehydrogenase activity in the corresponding test with DCPIP.

The enzymes of β-oxidation were detected in the peroxisomes from *Eremosphaera*, too. But in contrast to *Mougeotia*, activities of all three enzymes are also present in the mitochondrial fraction of this alga. The percentage of enoyl-CoA hydratase in the mitochondrial fraction is even higher than in the peroxisomal fraction. Therefore, in this case, trapping effects can be excluded and there is no doubt that this enzyme really is a constituent of the mitochondria.

The acyl-CoA oxidoreductase in the mitochondria of *Eremosphaera* shows a much higher activity with DCPIP than with O_2 and in that it is different from the peroxisomal enzyme of this alga. Therefore, it seems to be a dehydrogenase which is characteristic also for mitochondrial β-oxidation in animal tissues (9). Some activity of acyl-CoA oxidase as well as of 3-hydroxyacyl-CoA dehydrogenase detected in the *Eremosphaera* gradient at density 1.19 $g \cdot cm^{-3}$ may be due to trapped peroxisomes in the mitochondrial fraction.

The specific activities of the different enzymes are shown in Table I. Compared with corresponding data for spinach leaf peroxisomes (6), the algal peroxisomes contain only 12% to 35% of acyl-CoA oxidase or enoyl-CoA hydratase activity per mg protein. However, the specific activity of 3-hydroxyacyl-CoA dehydrogenase in the algae is almost 2-fold higher than in spinach leaf peroxisomes.

The data clearly demonstrate that enzymes of β-oxidation in algae are constituents not only of glyoxysomes (8) but also of peroxisomes of the leaf and unspecialized type, though in the latter two types of organelles the enzymes have much lower specific activities. For example, the activity of 3-hydroxyacyl-CoA dehydrogenase in *Euglena* glyoxysomes is more than 50 times higher than in the peroxisomes of *Mougeotia* and *Eremosphaera* (8). Almost the same is true for different enzymes in the glyoxysomes of *Ricinus communis* (2, 5), demonstrating that the β-oxidation pathway in the organisms possessing glyoxysomes is of much higher significance.

Considering the different results, it is becoming evident that the capability for degradation of fatty acids is a common feature of all different types of peroxisomes of algae as well as of higher plants.

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