Short Communication

Peroxidative Activity in Euglena gracilis

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ABSTRACT

Cell-free homogenates of Euglena gracilis contain very low levels of catalase activity as compared to higher plants and some other algae. Purified Euglena cytochrome c acts catalytically as a peroxidase. The observed catalytic activity of cytochrome c in extracts from heterotrophically grown cells was more than enough to account for the observed rates of hydrogen peroxide destruction. The peroxidative activity of Euglena cytochrome c was completely inhibited by 20 mM 3-amino-1,2,4-triazole.

There are at least two classes of microbodies in plant tissues; glyoxysomes present in germinating seedlings and peroxisomes from green leaves. The former class contains the enzymes of the glyoxylate cycle (1) and the latter the enzymes of the glycolate pathway (11). Both types usually contain flavin-linked oxidases, a fatty acyl-CoA dehydrogenase in the glyoxysome (6) and an α-hydroxy acid oxidase, usually glyoxysomal oxidase, in the peroxisome (9, 21). Hydrogen peroxide generated by these oxidases is destroyed by catalase, which is usually present in large amounts.

Microbodies of Euglena gracilis are also of two types. Glyoxysomes are present in cells grown heterotrophically on acetate (5, 13) and peroxisomes are present in phototrophically-grown cells (4, 15). However, cells grown on acetate would have no requirement for the enzymes of fatty acid oxidation, and peroxisomes from phototrophic cells do not contain the flavin mononucleotide-linked glycolate oxidase characteristic of higher plants but instead contain a glycolate dehydrogenase (4). In neither organelle is there any necessity for the presence of catalase (4), and early reports indicated its complete absence (12, 15). Recent reports, notably those of Brody and White (2, 3, 22) suggest that appreciable amounts of catalase are present in Euglena, particularly in acetate-grown cells. We wish to present evidence that the low levels of activity reported may be accounted for by the peroxidative activity of Cyt c or hemoproteins other than catalase.

MATERIALS AND METHODS

 Cultures of Euglena gracilis Klebs strain Z Pringsheim were grown organotrophically on Cramer and Myer's medium (7) supplemented with 0.2% (w/v) sodium acetate as carbon source, or phototrophically in 5% (v/v) CO₂-enriched air (15), while dark-grown cells were grown in Hutton's medium (14).

Cytochrome c was extracted from cells of Euglena grown on Hutton's medium (14). The cells were broken in a French pressure cell in 0.1 m phosphate buffer, pH 7. The cell-free extract was adjusted to pH 5 and centrifuged. The clear supernatant was then absorbed onto CM23 cellulose in 10 mM phosphate buffer, pH 7. The bound Cyt was eluted, absorbed onto CM52 cellulose, and eluted with 40 mM phosphate buffer, pH 7. The Cyt was recycled on the CM52 column successively in the oxidized and reduced forms until judged pure. The final purity index (E_{280}^{1cm}/E_{410}^{1cm}) was 0.88. The Cyt obtained is the soluble, mitochondrial type known as Cyt c-558 (17). The catalase-type activities of whole cell homogenates in a variety of conditions are shown in Table I. Comparable "catalase" activities are determined by methods used in earlier papers. The catalase-type activities of whole cell homogenates and of purified Cyt c were determined by the spectrophotometric method of Lück (16). Enzyme activities are expressed as μmoles of substrate transformed/min·mg of protein at 25 C. Cell-free extracts of acetate-grown cells were fractionated by isopycnic density gradient centrifugation on linear sucrose gradients as described previously (4).

Horse heart Cyt c was obtained from Sigma Chemical Co.

RESULTS AND DISCUSSION

The catalase-type activities of cell extracts of whole cell sonicates of Euglena grown under a variety of conditions are given in Table I. Comparable "catalase" activities are reported by Brody and White (3) on the assumption that one Luck unit is equivalent to 4 μmoles/min. If, however, the initial Η₂Ο₂ concentration used by Brody and White 12.5 μμoles/ml (as recommended in ref. 16) rather than 12.5 μmole/ml (as stated in ref. 22) the factor would be 4 μμoles/min. These activities are several orders of magnitude lower than those reported for other organisms—comparable specific activities for cell-free extracts of Chlorella and lower land plants are 15 to 92 μμoles/min·mg of protein (20). Although catalase accounts for about 10% of the total leaf peroxisome (9), the amount of catalase of similar specific activity in Euglena, even if concentrated entirely in the microbodies, would only amount to about 0.001% of the microbody protein.

It is possible that the observed activity in Euglena is the result of some other component with a weak peroxidative activity. One possible candidate is Cyt c. Early reports on the interaction of Cyt c with Η₂Ο₂ mentioned only that peroxide rapidly bleaches Cyt c, and that at low concentrations, peroxide may be used to reoxidise reduced Cyt. However, peroxide destruction during the bleaching of Cyt is several orders of magnitude higher than a 1:1 stoichiometry would require, and it seems reasonable to conclude that Cyt is acting catalytically as a peroxidase even if only transiently (Table I). Both the oxidized and reduced forms of Euglena Cyt c are equally effective, suggesting that the reoxidation of cytochrome is negligible at the high concentrations of Η₂Ο₂ used in the assay. Horse-heart Cyt also catalyses the destruction of Η₂Ο₂ (Table I).
Euglena cells, grown heterotrophically, contain approximately 1 µg of Cyt c/mg of whole cell-soluble protein. The measured catalase activity of Euglena cells may therefore be accounted for by the activity attributable to the presence of Cyt c, i.e., about 15 nmoles H₂O₂/min·mg of whole cell protein. The peroxidative activity of Euglena Cyt c was completely inhibited by 20 mM 3-amino-1,2,4-triazole and by 1 mM cyanide. Other mitochondrial Cyt, although present in smaller amounts, may also contribute to the observed activity. Catalase is a hemoprotein having a prosthetic group of the Cyt b type, so it might be expected that Cyt of the b-type might also have some peroxidative activity, although at a much reduced specific activity. Cyt f has also been shown to have weak peroxidative activity (8). The f-type Cyt of Euglena, c-552, will be present in light-grown cells. The appearance of this Cyt may explain increased peroxidative activity during regreening (2).

The cytochemical staining of microbodies using 3,3'-diaminobenzidine to localize catalase has produced variable results in algal species (19). White and Brody (22) report cytochemical staining of Euglena microbodies with 3,3'-diaminobenzidine stain under optimal conditions for the visualization of catalase, but even so, staining was observed in the absence of H₂O₂ with preparations from dark-grown cells (3). As pointed out by Seligman et al. (18), great care is required in the application of 3,3'-diaminobenzidine in ultrastructural cytochemistry because of the ability of 3,3'-diaminobenzidine to be polymerized by any hemoprotein. Moreover, the addition of H₂O₂ enhances the rate of 3,3'-diaminobenzidine oxidation by Cyt c or any other hemoprotein (18). The possibility of this occurring is probably enhanced in Euglena by the unusual structure of Cyt c, the heme of which has certain features in common with both protoporphyrin IX (heme B) and heme C (17). Because 3-amino-1,2,4-triazole inhibits the peroxidative activity in Euglena Cyt c, it cannot be assumed that any H₂O₂-dependent polymerization of 3,3'-diaminobenzidine is of necessity attributable to catalase. Although total levels of peroxidative activity are very low in Euglena, compared with some algae (10), the peroxidative activity of Cyt c more than accounts for the observed activity in Euglena extracts.

LITERATURE CITED


Table 1. Peroxidative Activities of Euglena Extracts and Cytochrome c

<table>
<thead>
<tr>
<th>Growth Conditions</th>
<th>Extract</th>
<th>&quot;Catalase&quot; specific activity</th>
<th>Rate of Cyt destruction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µmoles H₂O₂/min·mg protein</td>
<td>µmoles/min·mg</td>
</tr>
<tr>
<td>Organotrophic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>Crude cell extract</td>
<td>0.014 (0.004)</td>
<td>0.04</td>
</tr>
<tr>
<td>Light</td>
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<td>0.04</td>
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<td>Glucose medium</td>
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<td></td>
</tr>
<tr>
<td>Dark</td>
<td>Crude cell extract</td>
<td>0.016 (0.008)</td>
<td>0.04</td>
</tr>
<tr>
<td>Phototrophic</td>
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</tr>
<tr>
<td>Acetate medium</td>
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</tr>
<tr>
<td>Dark</td>
<td>Purified mitochondria</td>
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<td></td>
<td>Purified chloroplasts</td>
<td>0.045</td>
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</tr>
<tr>
<td></td>
<td>Purified microbodies</td>
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<td></td>
</tr>
<tr>
<td>Euglena Cyt c</td>
<td>oxidized</td>
<td>13</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>reduced</td>
<td>15</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Horse-heart Cyt</td>
<td>oxidized</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 Cells grown without aeration.