Note

Occurrence of Glycolate Dehydrogenase in Mitochondria and Microsomes in Streptomycin-Bleached Mutant of *Euglena gracilis* z[†]

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We have reported previously¹⁾ that glycolate dehydrogenase is present in both mitochondria and microbodies of *Euglena gracilis* grown on air under illumination and that the enzyme in the latter organelles is induced by conditions favorable for photorespiration but the enzyme in the former apparently has physiological functions independent of photorespiration. When *E. gracilis* is grown heterotrophically in the dark or light, the enzyme is present in only mitochondria.¹⁾ In the course of studying the glycolate metabolism in the streptomycin-bleached mutant of *E. gracilis*, which lacks chloroplasts, we have found that glycolate dehydrogenase occurs in mitochondria and microsomes but not in microbodies of this mutant.

The streptomycin-bleached mutant of *E. gracilis* z was obtained according to the method of McCalla²⁾ and was cultured in the Koren-Hutner's medium³⁾ in the dark. Cells reached the stationary phase of growth in about 4 days, and the cells grown for 7 days were

used throughout the experiments.

Procedures for linear sucrose density gradient centrifugation and assays of glycolate dehydrogenase and succinate dehydrogenase were described in a preceding paper.¹⁾ The optimum pH of glycolate dehydrogenase was determined in nitrogen atmosphere. Oxygen uptake was measured as reported by Tokunaga *et al.*⁴⁾ Antimycin A-insensitive NADPH cytochrome *c* reductase was assayed by the method of Williams and Kamin.⁵⁾ L- and D-Lactate dehydrogenases⁶⁾ were assayed spectrophotometrically by measuring the reduction of 2,6-dichlorophenolindophenol (DCIP) at 600 nm and 30°C. The reaction mixture (3 ml) contained 100 μ mol of sodium phosphate buffer (pH 6.8), 1 μ mol of DCIP, 10 μ mol of L- or D-lactate and enzyme solution. The reaction was started by the addition of L- or D-lactate. Protein was determined by the method of Lowry *et al.*⁷⁾

Figure 1 shows the distribution of the activity of glycolate dehydrogenase, as well as those of succinate dehydrogenase, a marker enzyme for mitochondria, and antimycin A-insensitive NADPH cytochrome *c* reductase, a marker enzyme for microsomes,⁵⁾ in fractions of 30% to 45% linear sucrose density gradient centrifugation of homogenate of the streptomycin-bleached mutant of *E. gracilis*. The succinate dehydrogenase activity showed a peak in Fractions 2 to 3 at an equilibrium density of 1.183, indicating that these represent mitochondria. The antimycin A-insensitive NADPH cytochrome *c* reductase activity was present in Fractions 2 to 3, 7 to 10 and 12 to 14. The activity in Fractions 2 to 3 is probably due to mitochondrial antimycin A-sensitive NADPH cytochrome *c* reductase; antimycin A has been reported not to inhibit completely this reductase.⁸⁾ Fractions 7 to #10 have equilibrium densities of 1.155 to 1.105 and represent

FIG. 1. Subcellular Distribution of Glycolate Dehydrogenase in the Bleached Mutant of *E. gracilis*. Ordinates, except for sucrose density, are plotted in nmol of substrates reduced/ml of fraction/min.

[†] Metabolism of Glycolate in *Euglena gracilis*. Part III.

microsomal fraction. Glucose-6-phosphatase, another marker enzyme for microsomes,⁹⁾ also showed activity in these fractions. The enzyme activity in Fractions 12 to 14 might be due to ferredoxin NADP reductase, which may be present in cytoplasm in the bleached *Euglena*,¹⁰⁾ but not due to cytochrome *c* reductase.

The glycolate dehydrogenase activity showed peaks in both the mitochondrial and microsomal fractions, when DCIP was used as the hydrogen acceptor, while the peak was found virtually in only the mitochondrial fraction when the enzyme activity was assayed with cytochrome *c* as the hydrogen acceptor, instead of DCIP. The results indicate that for the microsomal glycolate dehydrogenase, cytochrome *c* is not an efficient hydrogen acceptor. When the cell homogenate of the bleached mutant of *E. gracilis* was fractionated on 30% to 60% linear sucrose density gradient, the microbody fraction¹¹⁾ showed no activity for glycolate dehydrogenase at all.

Some properties of the glycolate dehydrogenase in mitochondria and microsomes are shown in Table I.

TABLE I. SOME PROPERTIES OF GLYCOLATE DEHYDROGENASE IN MITOCHONDRIA AND MICROSOMES

The protein concentration was 0.27 mg and 0.19 mg per reaction mixture in the mitochondrial and microsomal experiment, respectively.

	Specific activity (nmoles DCIP or cyt. <i>c</i> /mg protein/min)	
	Mitochondria	Microsomes
DCIP reduction		
Control	5.98	4.78
+1 mM KCN	1.11	1.76
Cytochrome <i>c</i> reduction	3.23	0.58
<i>K_m</i> for glycolate (mM)	0.19	0.44
Optimum pH	8.0	8.0

The mitochondrial enzyme reduces cytochrome *c* much more strongly than the microsomal enzyme, as described above; coupled with the glycolate oxidation, mitochondria took up oxygen. The apparent *K_m* for glycolate of the mitochondrial enzyme was slightly lower than that of the microsomal enzyme. The optimum pH of the enzyme in both organelles was identically 8.0. With DCIP as the hydrogen acceptor, the mitochondrial glycolate dehydrogenase was inhibited 81% by 1 mM KCN and the microsomal enzyme, 63%.

Graves *et al.*¹¹⁾ reported that the glycolate dehydrogenase activity in an ethanol-grown (dark), streptomycin-bleached mutant of *E. gracilis* var. *bacillaris* appeared to be due mainly to D-lactate dehydrogenase

from similar distributions of the two enzyme activities in their sucrose density gradient centrifugation. In our experiments both L- and D-lactate dehydrogenase activities were found in both mitochondria and microsomes in our bleached mutant of *E. gracilis* z. The activities of L- and D-lactate dehydrogenases in mitochondria were 12.1- and 28.1-fold higher, respectively, than the glycolate dehydrogenase activity; in microsomes they were 5.4- and 16.6-fold higher, respectively, than the glycolate dehydrogenase activity. The two lactate dehydrogenases in mitochondria were inhibited by only 11% by 1 mM KCN while those in microsomes by about 17%. These results strongly suggest that the glycolate dehydrogenase activity in mitochondria and microsomes of the bleached mutant of *E. gracilis* is not due to any lactate dehydrogenase but to the enzymes specific to glycolate. Nelson and Tolbert⁹⁾ reported that autotrophically grown *E. gracilis* contained L- and D-lactate dehydrogenases, resistant to KCN, in addition to glycolate dehydrogenase in the cell extract. Collins and Merrett¹²⁾ also found glycolate dehydrogenase and D-lactate dehydrogenase, distinct from each other, in *Euglena* mitochondria.

The glycolate-oxidizing enzyme is located in microbodies (peroxisomes) in higher plants,¹³⁾ while in mitochondria in some photosynthetic microorganisms.¹⁴⁻¹⁶⁾ The dual localization of this enzyme in mitochondria and microbodies in the wild strain of *E. gracilis*, and further in mitochondria and microsomes in the bleached mutant is highly interesting. The possible physiological functions of the enzyme in the latter two organelles are the subject of another paper which will be published elsewhere.

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