THE GLYCOLLATE OXIDISING ENZYME OF ALGAE

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1. Introduction

Unicellular green algae were thought to be incapable of oxidising glycollate produced during photosynthesis [1]. The phenomenon of glycollate excretion by several algae was explained as the result of an incomplete glycollate pathway in cells lacking a typical higher plant glycollate oxidase (glycollate: oxygen oxidoreductase E.C.1.1.3.1.).

Recently glycollate oxidising enzymes have been reported in several algae. Zelitch and Day [2] reported a glycollate dependent reduction of dichlorophenolindophenol by extracts of air grown *Chlorella* and *Chlamydomonas*, and Lord and Merrett [3] demonstrated glyoxylate formation from glycollate in extracts of *Chlorella* grown on 5% CO_2 in air. This algal enzyme was designated glycollate oxidase, although oxygen uptake was not measured. Nelson and Tolbert [4] have shown that the glycollate oxidising capacity of a strain of *Chlamydomonas* is regulated by the availability of CO_2 during growth. Cells grown on 1% CO_2 in air metabolised little glycollate but excreted it, whereas cells grown on air did not excrete glycollate. Growth on air derepressed a glycollate oxidising enzyme, previously designated glycollate: dichlorophenolindophenol (DCPIP) oxidoreductase [4], which reduced DCPIP but not oxygen.

The investigation reported here was undertaken to determine whether oxygen acts as the hydrogen acceptor during glycollate oxidation by extracts of *Chlamydomonas*, *Chlorella* and *Euglena*, and to determine the effect of CO_2 availability during growth on enzyme activity.

2. Materials and methods

The organisms used in this study were *Chlamydomonas reinhardtii* Dangeard (-) no. 90, *Chlorella pyrenoidosa* Cambridge Culture Collection, strain 211/8p and *Euglena gracilis* Z Klebs. *Chlamydomonas* was grown in the medium of Orth et al. [5], *Chlorella* in the medium of Goulding and Merrett [6], and *Euglena* on the photoautotrophic medium of Cramer and Myers [7].

All organisms were grown photoautotrophically at 25°C and light intensity 600 lumen/ft^2, and gassed with air or 5% CO_2 in air at the rate of 4 l/hr. Cells were harvested by centrifugation at 500 *g*, and disrupted by passage through an ice-cold French pressure cell [8] at 20,000 psi and the supernatant following at 10 min centrifugation at 10,000 *g* and 2°C was used in the enzyme assays. Glycollate: DCPIP oxidoreductase was assayed anaerobically and aerobically by following glycollate-dependent DCPIP reduction at 600 mμ on a Unicam S.P.500 spectrophotometer [2]. Protein was determined by the method of Lowry et al. [9].

Rates of oxygen consumption were measured on a Pt-Ag Rank Oxygen Electrode at 25°C in the dark and at 1200 lumen/ft^2 light intensity and recorded on a Rikadenki B241 two-pen recorder.

3. Results and discussion

The addition of glycollate to whole cell suspensions of air grown *Euglena* or 5% CO_2 in air grown *Chlorella* did not affect the rate of photosynthetic oxygen evolution or dark respiration. Cell free extracts

of the three organisms, grown on air or 5% CO_2 in air, showed no glycollate-dependent oxygen uptake in the light or in the dark.

However, the dye reduction assay showed all extracts to be capable of glycollate oxidation, thus indicating that oxygen did not function as the natural hydrogen acceptor for glycollate oxidation in these organisms. The specific activities of the enzyme for the three organisms grown on air or 5% CO_2 in air were calculated on the basis of dye reduction and are shown in table 1. These activities were sufficiently high to have ensured the detection of any glycollate-dependent oxygen uptake at the oxygen electrode sensitivity used here if oxygen had acted as hydrogen acceptor in the enzyme systems.

Table 1
Specific activities of glycollate: dichlorophenolindophenol oxidoreductase in algae grown in air, and in 5% CO_2 in air.

Organism	Specific activity[a]	
	Grown on air	Grown on 5% CO_2 in air
Chlamydomonas	0.198	0.045
Euglena	2.500	0.160
Chlorella	0.300	0.190

[a] Specific activity is defined as μmole glycollate oxidised/hr/mg protein.

The specific activities shown in table 1 were unchanged whether dye reduction was measured anaerobically or aerobically, which further suggested that the enzyme did not link to oxygen. Addition of FMN did not affect the rate in the cell extracts.

Glycollate: DCPIP oxidoreductase was barely detectable in extracts of *Chlamydomonas* grown on 5% CO_2 in air, whereas cells grown on air contained appreciable enzyme activity, as reported previously [4]. However, both *Chlorella* and *Euglena* grown on 5% CO_2 in air contained relatively high levels of enzyme. Growth on air increased the specific activity of the *Chlorella* enzyme only slightly, but resulted in a sixteenfold increase in specific activity for the *Euglena* enzyme.

The identity of the natural hydrogen acceptor, in lieu of oxygen, for glycollate oxidation in these algae is not known. It is assumed that the natural hydrogen acceptor was present in the crude *Chlorella* extracts used by Lord and Merrett [3] to demonstrate glyoxylate formation from glycollate aerobically in the absence of added electron acceptor.

Although the availability of CO_2 during growth has been shown to affect the level of glycollate: DCPIP oxidoreductase activity in *Chlamydomonas* and *Euglena*, *Chlorella* was little affected. Clearly some green algae contain high constitutive levels of enzyme. Until the identity of the natural hydrogen acceptor and factors controlling its availability are known, the regulation of glycollate metabolism in these algae cannot be fully understood. The nature of this natural acceptor in these algae is currently being investigated.

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