The Regulation of Glycollate Oxidoreductase with Photosynthetic capacity in *Chlamydomonas mundana*

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(Received 15 June 1970)

Unicellular green algae have been shown to contain an enzyme that catalyses the oxidation of glycollate to glyoxylate (Lord & Merrett, 1968; Zelitch & Day, 1968), but unlike the higher-plant glycollate oxidase (glycollate-oxygen oxidoreductase, EC 1.1.3.1) the algal enzyme does not link to oxygen (Nelson & Tolbert, 1969; Codd, Lord & Merrett, 1969). The identity of the natural hydrogen acceptor for glycollate oxidation in algae is, as yet, unknown.

The specific activity of this enzyme in *Chlamydomonas reinhardtii* is regulated by the CO₂ concentration during growth (Nelson & Tolbert, 1969). Cells grown on 1% CO₂ in air metabolized little endogenous glycollate but excreted this compound into the suspending medium, whereas growth on air depressed the enzyme when the cells did not excrete glycollate. Similarly the specific activity of glycollate oxidoreductase in *Euglena gracilis* is regulated by the availability of CO₂ during growth (Codd *et al.* 1969), and although the enzyme in a strain of *Chlorella pyrenoidosa* is relatively unaffected by CO₂ concentration this organism may not be typical of unicellular green algae in containing a high constitutive specific activity of the enzyme (Codd *et al.* 1969).

The regulation of the specific activity of glycollate oxidoreductase by CO₂ availability during growth is of note in relation to the report by Graham & Whittingham (1968) that photosynthetic capacity of *Chlorella* varied depending on whether the cells were grown in air or 5% CO₂ in air; high concentrations of CO₂ during growth decreased the rate of photosynthesis. To establish whether glycollate oxidoreductase and photosynthetic capacity are causally or coincidentally related, we determined enzyme activity in relation to photosynthetic CO₂ fixation in the obligate phototrophic green alga *Chlamydomonas mundana*.

This alga was selected because photoautotrophically grown cells fix CO₂ and evolve O₂ in the light at high rates, but the addition of acetate to the growth medium results in adaptive changes in the photosynthetic capacity of the cells (Russel & Gibbs, 1966). Cells grown in acetate medium are unable to

carry out typical green-plant CO₂ fixation because several enzymes of the Calvin cycle, including ribulose 1,5-diphosphate carboxylase, are repressed. Such cells carry out a photoassimilation of acetate to carbohydrate via the glyoxylate cycle (Eppley, Gee & Saltman, 1963). A unique feature of this organism compared with other green algae is that acetate specifically affects the formation of several Calvin-cycle enzymes without affecting total cell chlorophyll or the ability of the cell to carry out photosynthetic electron-transport reactions (Russel & Gibbs, 1966).

We transferred acetate-grown algae to inorganic medium to de-repress the synthesis of CO₂-fixing enzymes, to determine whether the synthesis of glycollate oxidoreductase was also affected by the regulatory system.

Methods. *Chlamydomonas mundana* (Boron strain) was grown at 32°C and 1500 lm/ft² continuous light in the acetate medium of Russel & Gibbs (1966). Cultures were gassed with 5% CO₂ in N₂.

Cells were harvested during the exponential growth phase by centrifuging for 5 min at 500g, washed once with inorganic medium and finally resuspended in inorganic medium or, when appropriate, inorganic medium containing 15 µg of cycloheximide/ml or 1 mg of chloramphenicol/ml. After transfer to inorganic medium cultures were gassed with air or 5% CO₂ in air, portions were removed at intervals and CO₂ fixation by whole cells and glycollate oxidoreductase activity in cell-free extracts were determined.

Photosynthetic CO₂ fixation was measured at 30°C and 1000 lm/ft² light-intensity in Warburg flasks containing 2 ml of a cell suspension in 10 mM-potassium phosphate buffer, pH 7.0 (containing the equivalent of 100–150 µg of chlorophyll/ml). The reaction was started by the addition of 0.25 ml of NaH¹⁴CO₃ solution (containing 40 µmol, 4 µCi) and was terminated after 10 min by the addition of 0.5 ml of 0.5 M-HCl. Chlorophyll concentration was determined by the method of Arnon (1949). Cell-free extracts were prepared by sonication (30 s maximum output from MSE model 60W ultrasonic oscillator) of cells suspended in 10 mM-potassium

Fig. 1. Changes in photosynthetic capacity (a) and glycollate oxidoreductase activity (b) in *C. mundana* transferred from acetate medium to inorganic medium that was gassed with air (●), was gassed with 5% CO₂ in air (○), contained 15 μg of cycloheximide/ml and was gassed with air (Δ) or contained 1 mg of chloramphenicol/ml and was gassed with air (□).

phosphate buffer, pH 7.0, and kept at 1°C. Cell debris was removed by centrifugation for 10 min at 10000g and the supernatant used immediately in enzyme assays. Glycollate oxidoreductase activity was measured aerobically (Lord & Merrett, 1970) and soluble protein determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Results and discussion. Transfer of *C. mundana* from acetate to inorganic medium that was gassed with air or 5% CO₂ in air resulted in a rapid increase in the capacity of the cells to fix CO₂ (Fig. 1a), as reported by Russel & Gibbs (1966). The effect of two protein-synthesis inhibitors, cycloheximide and chloramphenicol, was to prevent this increase in photosynthetic capacity in cells gassed with air (Fig. 1a). The results suggest that transfer to inorganic medium resulted in the synthesis *de novo* of the enzymes involved in CO₂ fixation. The failure of acetate-grown cells to fix CO₂ was therefore apparently not due to acetate or some metabolite derived from acetate inhibiting enzyme action. Similarly transfer to inorganic medium brought about the synthesis *de novo* of glycollate oxidoreductase (Fig. 1b). A similar increase in glycollate oxidoreductase activity on transfer of cells from acetate to inorganic medium was obtained when enzyme activity was expressed on a protein basis. The photosynthetic capacity and glycollate oxidoreductase activity of cells kept in acetate medium did not alter during the experimental period.

Cycloheximide and chloramphenicol at the concentrations used here have been shown to inhibit preferentially cytoplasmic and chloroplasmic enzyme synthesis respectively in *Euglena gracilis* (Smillie, Graham, Dwyer, Grieve & Tobin, 1967). Clearly these concentrations do not result in this specific mode of action in *C. mundana*, as both inhibitors affect photosynthetic CO₂ fixation (Fig. 1a) and presumably the synthesis of chloroplast proteins.

The glycollate pathway has been shown to operate during the growth of *Chlorella* on exogenous glycollate (Lord & Merrett, 1970) and also at certain stages of the division cycle in synchronized cultures of *Euglena* (G. Codd, unpublished work). The synthesis of glycollate oxidoreductase at the same time as CO₂-fixing capacity increases further suggests that the glycollate pathway has a functional role in the further metabolism of photosynthetically fixed carbon in green algae.

We thank the Science Research Council for the award of a research studentship (to J. M. L.).

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