Short Communication

The Effect of Light Quality on Glycolate Formation and Excretion in Algae

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The excretion of glycolate by unicellular green algae is well documented (17) and is enhanced by low CO2 partial pressure, high O2 partial pressure, and high light intensity. Becker et al. (1) investigated the effect of light quality on glycolate excretion during photosynthesis in Chlorella. Cells grown in white light excreted glycolate when exposed to high intensity white or red light, but no glycolate excretion was observed in blue light. These workers suggested that in blue light the glycolate formed is further metabolized by the cell via glyoxylate to glycine and other amino acids utilized during protein synthesis, which has been suggested to be predominant in blue light. The glycolate pathway is well documented in higher plants (15), and this pathway has been shown to operate in Chlorella utilizing exogenous glycolate (11) and at certain stages of the division cycle in Euglena (G. A. Codd and M. J. Merrett, unpublished results). The glycolate oxidizing enzyme of algae, originally reported to be an oxidase (10, 19), was later shown to be a dehydrogenase (3, 13). The flavoprotein glycolate oxidase of tobacco is inhibited by blue light (16), and if the algal enzyme also contained flavin mononucleotide as the coenzyme, then enhanced rather than diminished glycolate excretion by algae in blue light would be expected. This being so, an alternative hypothesis to explain the failure of algae to excrete glycolate in blue light would be that blue light does not support glycolate biosynthesis. We have investigated the effect of light quality on glycolate formation by forcing the excretion of glycolate formed with a-hydroxy-2-pyridinemethane sulfonate which inhibits glycolate metabolism in higher plants (18) and algae (12) in the dark, and then exposed to 16,000 lux white light provided by a photoflood lamp or 10,000 lux intensity light transmitted by a blue (Chance blue filter O.B.10) or a red (Locarte red filter L.F. 10) glass filter. The transmission of both these filters was determined spectrophotometrically by us; the blue filter provided light of wave length between 350 and 530 nm and the red filter light of wave length above 570 nm. Throughout the entire experiment the cells were gassed with 100% O2. At appropriate times, samples were removed into ice-cold centrifuge tubes, the cells spun rapidly down, and the supernatant decanted. The glycolate content of the supernatant was determined by the method of Calkins (2).

RESULTS AND DISCUSSION

The strain of C. pyrenoidosa used in this work does not excrete glycolate in white light under the optimal conditions described in "Materials and Methods" when grown on 5% CO2 in air (6), whereas E. gracilis does so (G. A. Codd and M. J. Merrett, unpublished). That Chlorella forms glycolate under such conditions is known since excretion can be forced in the presence of a-HPMS (12).

Both organisms contain appreciable glycolate dehydrogenase activity when grown on 5% CO2 in air (3), but glycolate excretion is observed with Euglena but not Chlorella because Euglena forms much more glycolate on a dry weight basis than Chlorella. Growing the cells on air does not markedly increase the level of glycolate dehydrogenase in Chlorella, whereas this resulted in a 16-fold increase in activity for the enzyme in Euglena (3), and in such cells the glycolate formed is metabolized rather than excreted (G. A. Codd and M. J. Merrett, unpublished).

When Euglena cells were illuminated by blue light, under conditions favoring glycolate formation, no excretion occurred (Fig. 1). The addition of a-HPMS did not force excretion indicating that glycolate biosynthesis was not occurring.

In red light Euglena formed and excreted glycolate, and the rate of excretion was not significantly increased in the presence of a-HPMS (Fig. 1).

The failure to synthesize glycolate in blue light was confirmed by repeating these experiments with Chlorella. This alga did not excrete glycolate in either red or blue light, but the addition of aHPMS forced excretion in red light but not in blue light (Fig. 2).

The results of Becker et al. (1) are best explained as a failure of algae to form glycolate rather than a further enhanced metabolism of this compound in blue light. The results reported here also explain why Hauschild et al. (8) found that in Chlorella vulgaris grown on 5% CO2 in air in white light, photosynthetic 14CO2 fixation carried out in blue or red supplemented with blue light resulted in a decrease in the radioactivity incorporated into glycolate and glycine, compared with the incorporation into these compounds in red light.

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Hess and Tolbert (9) grew algae on 0.2% CO$_2$ in red or blue light and investigated the effect of light quality on the distribution of $^{14}$C among the products of photosynthesis.

When *Chlorella* or *Chlamydomonas* grown in blue light were exposed to $^{14}$CO$_2$ in either blue or white light for 3 to 10 min, glycolate was the major radioactive product and contained 30 to 36% of the total soluble $^{14}$C fixed.

Algae grown in red light incorporated less than 3% of the total soluble $^{14}$C into glycolate. These algae grew rapidly in red or blue light only after several days of relatively slow growth, and it is probable that during this adaptation period changes occurred in the metabolism of the cells which account for the differences between these cells and cells grown in white light.

The results presented here provide evidence for the major involvement of photosystem I, driven by red light, during glycolate biosynthesis by the organisms used. Such a concept is not inconsistent with the current hypotheses of the mechanism of glycolate formation during photosynthesis.

Coombs and Whittingham (4) suggested that low CO$_2$ concentrations and high light intensity could result in a decreased turnover of NADP/NADPH and an increased rate of ferredoxin photoreduction respectively. These workers further suggested that increasing O$_2$ concentrations might increase the rate of oxidation of reduced ferredoxin by molecular O$_2$ in a "Mehler" type reaction. Hydrogen peroxide thus formed may function as an oxidant in glycolate formation from its immediate carbon precursor. More recently Plaut and Gibbs (14) have suggested a possible role for photosystem I in glycolate formation from their work with isolated spinach chloroplasts.

**LITERATURE CITED**


