Short Communication

Absence of Phytochrome Participation in Chlorophyll Synthesis in Euglena

MICHAEL E. BOUTIN AND RICHARD M. KLEIN
Department of Botany, University of Vermont, Burlington, Vermont 05401

There appear to be three phases in chlorophyll synthesis in Euglena (11). An initial, rapid conversion of a small pool of protochlorophyll(ide) to chlorophyll is followed by a short lag phase during which the protochlorophyll(ide) pool is replenished; the third phase is that of rapid and sustained chlorophyll synthesis. Since the initial pool of protochlorophyll(ide) is smaller than would be expected from an intermediate accumulating behind a dark-blocked step (11), Gassman and Bogorad (5) suggested that in vascular plants the concentration of its precursor, δ-amino levulinic acid, may be limiting, but there is no proof that ALA² is limiting in Euglena. In higher plants, many workers (2, 4, 12) found that the enzymatic synthesis of protochlorophyll(ide) is activated by phytochrome. This study was designed to evaluate the possible role of phytochrome in chlorophyll synthesis in dark-grown Euglena.

MATERIALS AND METHODS

Euglena gracilis Klebs var. bacillaris Pringsheim, obtained from Dr. Seymour Hutner, was grown in an acid organotrophic medium (6) in darkness at 24 to 26 °C for at least a month with transfers under a green safelight at 5 day intervals before being used for an experiment (3). Although Dr. Carl A. Price (personal communication) found that ALA may not penetrate into Euglena cells, ALA was added to a final concentration of 1 μM to decrease the possibility that ALA might be limiting chlorophyll synthesis. Cell counts were made with a Sedgwick-Rafter plankton counter (8) after killing the cells with 1.5% (v/v) formaldehyde. Chlorophyll was determined by the method of Arnon (1) after extraction into 80% (v/v) acetone and filtration through glass fiber filter disks. Results are expressed as mg total chlorophyll/cell.

Red light (peak at 660 nm, 1270 μw/cm²) and far red light (peak at 720–730 nm, 1590 μw/cm²) were obtained with solid filter-liquid filter combinations (9). White light (200 ft-c) was supplied by cool white fluorescent lamps cooled with a fan.

RESULTS AND DISCUSSION

The rate of chlorophyll synthesis in dark-grown Euglena was followed in continuous white light following a 24-hr dark period after transfer to the experimental flasks (Fig. 1). There was a lag phase of 18 hr followed by sustained chlorophyll synthesis for at least 60 hr of illumination. These results are in substantial agreement with those of Stern et al. (11) and others.

For studies of short term irradiation with red light on chlorophyll synthesis, 5 ml of dark-grown cells were transferred 24 hr before any irradiation to 50 ml Erlenmeyer flasks con-
taining 20 ml of medium. The flasks were irradiated from below for 15 min with sufficient red light (1.14 joules/cm²) to convert phytochrome completely to the Pfr form (10). The cells were then continuously illuminated with white fluorescent light. Preillumination with red light had no effect on cell numbers or on the concentration of chlorophyll per cell (Fig. 2). Identical results were obtained in a medium (11) that does not support cell division.

The potentiation effect of Holowinsky and Schiff (7) was also studied. Dark-grown cells were exposed to red light for 15 min, returned to darkness for 12 hr and subsequently exposed to continuous white light. Under these conditions, there was no statistically significant potentiation effect of either cell numbers or on the concentration of chlorophyll per cell.

To complete the study, dark-grown cells were first illuminated with red light for 15 min immediately followed by far red light for 15 min (1.50 joules/cm²). Although there was a slight increase in chlorophyll per cell in red light-treated populations and a decrease in chlorophyll synthesis in far red light-treated cells, the observed differences were not statistically significant. Our results confirm the conclusion of Holowinsky and Schiff (7) that phytochrome may not be involved in chlorophyll synthesis in *Euglena*.

LITERATURE CITED


