Quantitative and compositional changes in monogalactosyl and digalactosyl diglycerides during light-induced formation of chloroplasts in *Euglena gracilis*

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ABSTRACT The formation of chloroplasts in dark-grown cells of *Euglena gracilis* was induced by exposing the cells to constant illumination. Following a lag, the cells accumulated chlorophyll and galactosyl diglycerides simultaneously at almost linear rates.

The monogalactosyl diglyceride content rose from approximately 2 μmoles in 100 mg of dark-grown cells to 27 μmoles in fully green cells; the digalactosyl diglyceride content increased from 1 μmole to 11 μmoles. The digalacto compounds increased more rapidly than the monogalacto compounds at first, but their rate of accumulation began to diminish long before greening of the cell was complete. The sole exception was the digalactosyl diglyceride fraction that contained hexadecadienoic (16:2) fatty acid. This fraction increased continuously during greening. As accumulation of the digalacto compounds diminished, that of the monogalacto compounds increased. Towards the end of greening, the major fatty acids were 16:2, 16:3, 16:4, 18:2, and 18:3 in the monogalacto and 16:2 in the digalacto compounds.

The results of this study suggest that monogalactosyl and digalactosyl diglycerides that contain particular fatty acid components have a function in the assembly of chloroplasts.

KEY WORDS *Euglena gracilis* · chloroplasts · development · galactosyl diglycerides · fatty acids · composition · photosynthesis

THE CHLOROPLASTS of photosynthesizing cells are known to have a large quantity of galactosyl diglycerides (1, 2) but, thus far, the function of these compounds has not been unveiled. The present study was designed to furnish clues to their specific role. The study deals with kinetics of accretion of chlorophyll and of galactosyl diglycerides in the model organism *Euglena gracilis* while it forms chloroplasts under constant light.

Light-grown green cells of *Euglena gracilis* have a large store of galactosyl diglyceride (3). Dark-grown cells have a small amount, even though they have no measurable amount of chlorophyll (3). During the formation of chloroplasts in dark-grown cells exposed to light, lipid-bound galactose accumulates in the cells concurrently with chlorophyll (4).

This report identifies the lipid-bound galactose as consisting mostly of monogalactosyl and digalactosyl diglycerides, and indicates that rates of accretion in the greening cell differ widely among the various molecular species of galactosyl diglycerides involved.

The formation of chloroplasts has been induced by light at an intensity lower than that used before (4). Under these conditions, chloroplasts form more slowly and transitory changes are more easily detected. Moreover, local increases in temperature are minimized. These tend, in our experience, to cause the development of visibly abnormal chloroplasts.

MATERIALS AND METHODS

Several generations of *Euglena gracilis* were grown in total darkness at 25°C in a complete, defined medium as

Fatty acids are designated by number of carbon atoms: number of double bonds.

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described previously (4). Approximately 10 g of cells in 2-liter batches of culture medium were harvested after 11 days of growth. The cells, washed twice in distilled water at 4°C, were suspended in 500 ml of a mineral medium (0.01 M KH_2PO_4, 0.01 M $MgCl_2$) in which they were subjected to continuous fluorescent illumination (15-watt Cool White tubes) at approximately 30 foot-candles in a temperature-controlled cabinet at 25°C. Cultures were shaken to insure uniformity, and 10-ml samples were withdrawn periodically under sterile conditions. The cells were centrifuged, washed in distilled water, and collected, all at 4°C. They were extracted with 20 volumes of chloroform–methanol 2:1 in a Waring Blendor (2 min, 10°C), and the extract was dialyzed against running tap water at 4°C overnight in the dark. The chloroform layer of the dialysate was collected, and dissolved water was removed with anhydrous Na_2SO_4. The chloroform solution was concentrated under vacuum at room temperature to a suitable volume (5 ml). The pure monogalactosyl and digalactosyl diglyceride fractions were isolated from this solution and analyzed as described previously (3).

RESULTS

Fig. 1 shows the increase in cellular chlorophyll content, the corresponding increase in total lipid-bound galactose, and the increases in the individual monogalactosyl and digalactosyl diglyceride fractions during active greening of *Euglena* cells at a constant level of illumination. After a lag of about 40 hr, total lipid-bound galactose increased continuously at an almost linear rate over the next 125 hr. After this time, cell contents of both chlorophyll and galactosyl diglyceride began to level off. A description of the galactosyl diglycerides during this later, stable period is provided elsewhere (3).

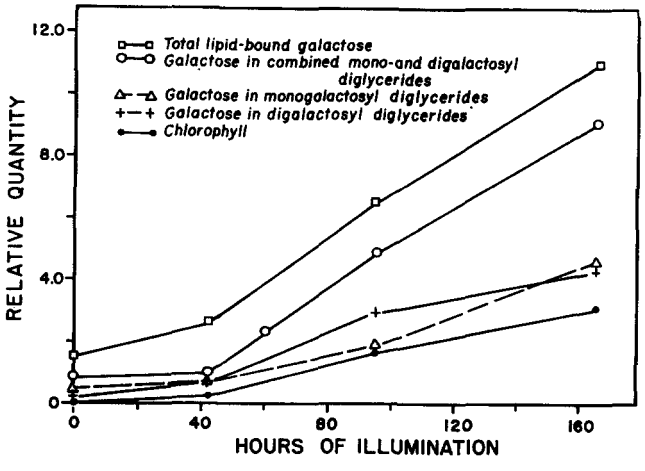

FIG. 1. Changes in the relative quantities of total lipid-bound galactose, monogalactosyl diglycerides, digalactosyl diglycerides, and chlorophyll in cells of *Euglena gracilis* grown in the dark in a complete medium, and then exposed to constant illumination in a mineral medium. The galactose values are expressed as milligrams per 100 mg of cells (approximately) contained in replicate samples of exactly 10 ml of culture. Chlorophyll is measured in terms of absorbance at 668 mμ in chloroform.

As shown in Fig. 1, not all of the cellular galactolipid was found in the monogalactosyl and digalactosyl diglyceride fractions. Before the onset of greening, when cellular monogalactosyl and digalactosyl diglyceride levels were low, the galactose in these compounds comprised only half of the total lipid-bound galactose in the cell. Although the quantity of galactosyl diglycerides increased during active greening, the quantity of lipid-bound galactose not accounted for as monogalactosyl and digalactosyl diglycerides remained constant. Consequently, increasingly larger proportions of total lipid-bound galactose were found in the galactosyl diglycerides, until a level of approximately 80% was reached and maintained.

At first, the rate of accumulation of galactose in digalactosyl diglycerides was greater than that in monogalactosyl diglycerides. Later, the rate of accumulation of galactose in the digalacto compounds diminished and that in the monogalacto compounds increased until at the end of the active greening period there were roughly equal amounts of galactose in the two lipid fractions. However, since the monogalacto compounds have half the galactose content of the digalacto compounds, on a molar basis, the level of cellular monogalactosyl diglyceride was always greater than that of digalactosyl diglyceride. At the end of the period of active greening, the molar ratio of digalactosyl to monogalactosyl diglyceride was 0.5.

Marked changes in the composition of the fatty acids of the galactosyl diglycerides accompanied chloroplast formation. The major changes in the fatty acid composition of the galactosyl diglycerides are shown in Fig. 2. In the monogalactosyl diglycerides (Fig. 2, left), 16:0 and 18:0 fatty acids dropped to trace concentrations, while the percentages of 16:1 and 18:1 fatty acids diminished by two-thirds. At the same time, the percentages of 16:2, 16:4, and 18:2 fatty acids increased to a point where they constituted more than half of the total fatty acid complement of the monogalactosyl diglyceride fraction of the fully green cell. In the digalactosyl diglycerides (Fig. 2, right), the percentages of 16:0, 16:1, 18:0, 18:1 fatty acids also diminished greatly, while 16:2 and 18:2 showed major increases in percentage. The rates of increase in the absolute quantity of each fatty acid species in the mono- and the digalactosyl diglyceride fractions of the greening cells are shown in Fig. 3. The figure indirectly gives the total amounts of mono- and of digalacto compounds present in the cell as the chloroplasts form.

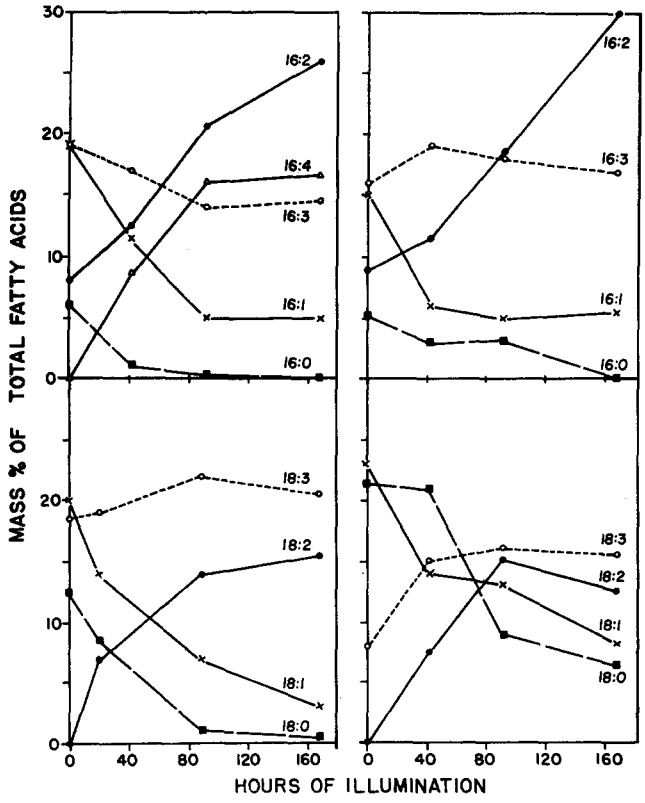

FIG. 2. Changes in the composition of the 16-carbon and 18-carbon fatty acids in the galactosyl diglycerides of greening cells of *Euglena gracilis* exposed to constant illumination. Values for the monogalactosyl fraction are shown on the left, for the digalactosyl fraction on the right. The values were calculated from gas–liquid chromatographic peak areas before and after hydrogenation of the fatty acids.

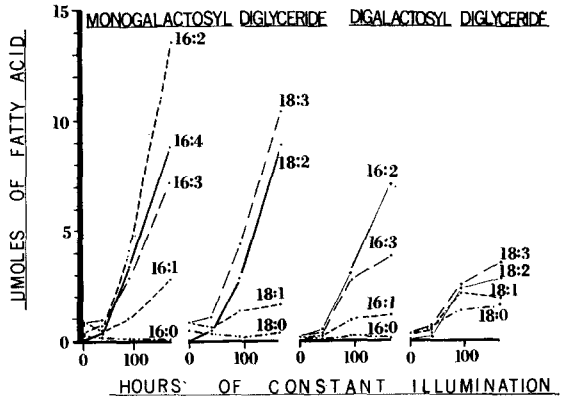

FIG. 3. Changes in the absolute amount of each fatty acid species in the monogalactosyl and in the digalactosyl diglycerides of dark-grown cells of *Euglena gracilis* exposed to constant illumination in a mineral medium. The values are expressed as micromoles of fatty acid per 100 mg of cells (approximately) in replicate samples of exactly 10 ml of culture. These data are calculated from those given in Figs. 1 and 2.

During the lag which comes before the period of rapid rise in chlorophyll (Fig. 1), 16:0, 16:1, 18:0, and 18:1 fatty acids tend to disappear from the monogalactosyl fraction. The amounts of all the other kinds of fatty acids either remain unchanged or rise slightly.

When molecules of chlorophyll begin to multiply quickly (Fig. 1), a sizable accretion of galactosyl diglycerides begins, with the exception of those containing 16:0 and 18:0 fatty acids. After the cells have undergone greening for approximately 45 hr, a trend towards levelling of the cellular content of digalactosyl diglycerides becomes discernible. Only those digalactosyl diglycerides that contain 16:2 continue to accumulate at the same rate (Fig. 3). Meanwhile, all of the fatty acids in the monogalactosyl diglycerides show continuous increase, with the exception of 16:0 fatty acid (which can no longer be found) and 18:0 and 18:1 which do not show much real change in amount.

DISCUSSION

Dark-grown cells of *Euglena gracilis* contain a small amount of galactosyl diglycerides (Fig. 1). In the monogalactosyl fraction, 16:1, 18:1, 16:3, and 18:3 are found in equal proportion and are the major fatty acid components (Fig. 2). No 16:4 or 18:2 fatty acids are present; these fatty acids increase, with greening of the cell, from zero concentration to between 15 and 20% of the fatty acids in the monogalactosyl diglycerides, as does 18:2 fatty acid in the digalactosyl diglycerides. Dark-grown cells of *Euglena gracilis* have neither chlorophyll (4) nor structures that one can call chloroplasts (5). The results in Fig. 3 suggest that galactosyl diglycerides, in small amounts and with acids of a lower degree of unsaturation, occur in membrane systems other than in chloroplasts.

Benson (6), and Kates and Volcani (7) have proposed that galactosyl diglycerides with a rich complement of unsaturated fatty acids may be required for the development of chloroplast lamellae with properly oriented chlorophyll molecules and with particular permeability properties. Under the conditions of the present study, 16:2 is the superabundant fatty acid in the galactosyl diglycerides of the green cell, with more than 13 μmoles/100 mg of cells in the monogalactosyl fraction and 7 μmoles/100 mg in the digalactosyl fraction (Fig. 3). It is the only fatty acid present at a major level in the digalactosyl diglycerides. Other fatty acids found in major amounts (7–11 μmoles) in the monogalactosyl diglycerides are, in descending order, 18:3, 16:4, 18:2, and 16:3. Minor amounts (<4 μmoles, >1 μmole) of 16:1 and 18:1 fatty acids are found in the monogalactosyl diglycerides, and of 16:1, 16:3, and 18-carbon fatty acids in the digalactosyl diglycerides. Only trace amounts are found of 16:0 and 18:0 fatty acids in monogalactosyl diglycerides, and of 16:0 in the digalactosyl diglycerides.

The results of this study suggest that the complex route of assembly of the chloroplast depends not only on chloro-

phyll (8), but also on galactosyl diglycerides with specific fatty acids. Of the four major classes of lipid in *Euglena gracilis* [wax esters (9), phospholipids, sulfolipids, and galactosyl diglycerides], only sulfolipids and galactosyl diglycerides show a great increase during the light-induced greening of dark-grown cells. Phospholipids decline somewhat, then regain their starting level; wax esters drop to traces (4). The content of galactosyl diglycerides increases much more than does that of sulfolipids.

At the lower level of illumination (30 foot-candles) used in these experiments, the accumulation of chlorophyll in the cell not only is slower, but appears to have somewhat different kinetic features from that at the higher level of illumination (90 foot-candles) used previously (4). Yet the increase in lipid-bound galactose starts concurrently with that in chlorophyll at both intensities of light and, in each case, the rate of increase is almost linear.

Total lipid-bound galactose accumulates at a rate proportional to the rate of chlorophyll increase; its amount is not a function of the chlorophyll level. Thus it seems probable that galactosyl diglycerides take part in chloroplast formation and do not pile up as mere end products of a metabolic process that occurs within the mature chloroplast.

Although the total amount of galactose bound to lipid increases at a continuous rate, that in each of the lipid subfractions does not. Accumulation of the digalactosyl fraction slackens after 50 hr of active greening of the cell (Fig. 1), at which time there is a rise in the rate of accumulation of the monogalactosyl fraction. The monogalacto compounds uniformly rise at a greater rate and correspondingly, the digalacto compounds uniformly accumulate in decreased amounts, with the sole exception of the digalactosyl diglyceride fraction containing 16:2 fatty acid (Fig. 3).

The results of this study indicate that accumulation of monogalactosyl and digalactosyl diglycerides with specific fatty acid components may be required in the building of functional chloroplast structures in *Euglena gracilis*.

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