LIGHT-INDEPENDENT STOICHOMETRY OF GALACTOSYL DIGLYCERIDE AND CHLOROPHYLL ACCRETION DURING LIGHT-INDUCED CHLOROPLAST MEMBRANE SYNTHESIS IN EUGLENA

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SUMMARY: In photobiotic Euglena gracilis, chlorophyll biosynthesis takes place at a rate which is a direct function of light intensity. There is stoichiometry between chlorophyll accumulation and that of galactosyl diglycerides. This stoichiometry remains relatively invariant, regardless of the wide changes in chlorophyll content brought about in response to controlled variation of light intensity. These findings suggest that the formation of chlorophyll-containing photoreceptor membranes may be paced and stabilized by concurrent synthesis of galactosyl diglycerides.

The mechanism underlying light-induced chloroplast membrane assembly in plants and eukaryotic protists like Euglena remains an unsolved problem. These higher photosynthetic organisms, if dark-grown, do not have functional chloroplasts. Light induces presumably complex reactions which lead to the biosynthesis of chlorophyll and the assembly of functional chloroplasts. Synthesis of sulfoquinovosyl diglyceride (1), one of the putative chloroplast lamellar lipid components appears to precede the appearance of measurable chlorophyll (2) in light-exposed etiolated organisms. So does, to some degree, the conversion of photochlorophyllide to chlorophyllide (3), the hydrophilic light-absorbing moiety of chlorophyll. Continuous light at an intensity of 150 ft-c appears to be optimum for the induction of chlorophyll synthesis (4) in dark-grown Euglena. At higher intensities, no increase in rate of chlorophyll synthesis is observed. An initial "latent" interval displays intracellular morphological changes (5) and suggests that a degree of development of the membranous structure of the protochloroplast takes place before the appearance of chlorophyll which, itself, is a complex lipid molecule.

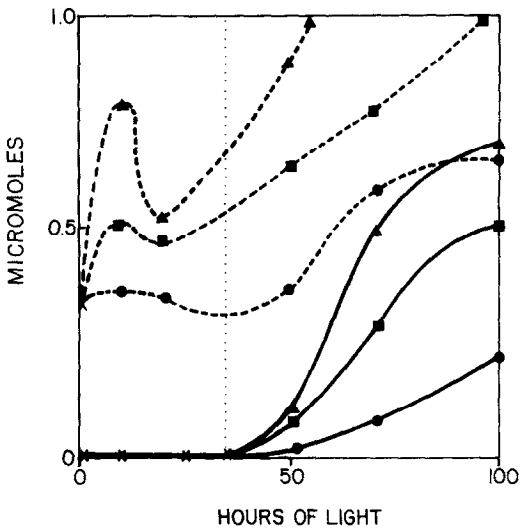

Figure 1: Early synthesis of galactosyl diglycerides (---) and chlorophyll (——) in etiolated Euglena upon exposure to light. Values are expressed as content per sample (4 x 10^7 organisms). Conditions are the same as in Materials and Methods. Maximum standard error: ± 6%. Each point represents mean value obtained for three independent cultures. ●, 10 ft-candles; ■, 50 ft-candles; ▲, 120 ft-candles.

We have suggested that galactosyl diglycerides, the major lipid component along with chlorophyll in functional chloroplast membranes of higher organisms, may serve the function of a containing matrix for chlorophyll molecules (6) in chloroplast membranes. For the present study, Euglena were kept under photobiotic conditions, i.e. in a medium devoid of a source of nitrogen and metabolic energy and exposed to controlled light intensities. This experimental arrangement permits regulation of the rate of chlorophyll accretion and reveals that increase in chlorophyll is always approximately stoichiometric with galactosyl diglyceride.

MATERIALS AND METHODS

Growth of organisms. Euglena gracilis, strain Z was grown in the dark in a complete medium (7). Upon reaching the beginning of the stationary phase of growth, the organisms were harvested by low speed centrifugation in the dark at room temperature. They were washed and suspended in 10 mM KH_2PO_4 - 10 mM $MgCl_2$. Each suspension contained approximately 4 x 10^7 organisms per liter of solution in a 2-liter round bottom flask.

The flasks, at 25°C, were exposed to continuous fluorescent light (cool white) at the intensities shown below. Light intensity was measured with a Weston light meter at the surface of the suspension medium. Samples were removed at intervals. Growth of the organisms and collection, suspension and the removal of samples were all done under sterile conditions.

Extraction of lipids. The organisms were harvested by centrifugation at 1000 x g for 3 minutes. Twenty volumes of ice-cold chloroform-methanol, 2:1, v/v, were added with mixing and the slurry was placed at -20°C for 15 hours in the dark. This arrangement completely extracts all of the galactosyl diglyceride and chlorophyll. The extract was then brought to room temperature and filtered. Aliquots were removed, the solvents evaporated with a stream of nitrogen, and the samples analyzed for chlorophyll and for galactosyl diglyceride as described (7). Experiments were performed in triplicate on separate cultures.

RESULTS

The data in Fig. 1 suggest a small but rapid initial accumulation and dissipation of galactosyl diglycerides immediately after exposure of the organisms to light. The galactosyl diglyceride level increases again along with the appearance of major amounts of chlorophyll and in close stoichiometry with it. Stoichiometry is displayed at all light intensities, even though the rate of chlorophyll biosynthesis varies considerably in response to the intensity of light (Table I). Figure 2 shows the rate of appearance of galactosyl diglyceride relative to chlorophyll. Since there is a small amount of pre-existing galactosyl diglyceride, but no significant quantity of chlorophyll, the ratio of galactosyl diglycerides to chlorophyll descends asymptotically from infinity. A turning point is discernible when the organisms have produced one molecule of chlorophyll for somewhat more than two of pre-existing galactosyl diglycerides. Beyond this point, as shown in Table I, the rate of chlorophyll synthesis becomes a direct function of light intensity. A rapid biosynthesis of galactosyl diglyceride now commences and keeps a pace slightly in advance of chlorophyll (Figure 2), and thus it is also apparently proportional to light intensity. There is no evidence that galactosyl diglyceride synthesis is a light requiring process. The data in Table I show that at any given time, there are wide differences in chlorophyll content of the organisms, depending upon light intensity. Galactosyl diglycerides show corresponding-

Chlorophyll Accretion in Etiolated Euglena

Exposed to Continuous Light Under Photobiotic Conditions

		Intensity Foot-candles	Time Hours	Chlorophyll μmoles x 10^7/cell
1.	a	10	50	5
	b	10	75	27
	c	10	150	100
	d	10	275	150
	e	10	350	165
2.	a	20	50	25
	b	20	75	55
	c	20	150	140
	d	20	275	200
	e	20	350	210
3.	a	120	50	45
	b	120	75	150
	c	120	150	250
	d	120	275	330
	e	120	350	360

Approximately 10^7 dark-grown organisms were suspended in 10 mM KH_2PO_4 - 10 mM $MgCl_2$ at 25°C and exposed continuously to "cool-white" fluorescent light. Chlorophyll was determined spectrophotometrically in cell extracts. Maximum standard error: ± 6%. Each point represents mean value obtained for three independent cultures.

ly wide variety, and the chlorophyll-glycolipid molecular ratio remains independent of light intensity (Figure 2). These data suggest that galactosyl diglyceride and chlorophyll incorporation into the membrane stabilizes these molecules as a structural entity.

Most, if not all, of the galactosyl diglycerides and chlorophyll molecules in the cell are components of chloroplast membranes. Our findings suggest that assembly of galactosyl diglyceride-chlorophyll combinants in

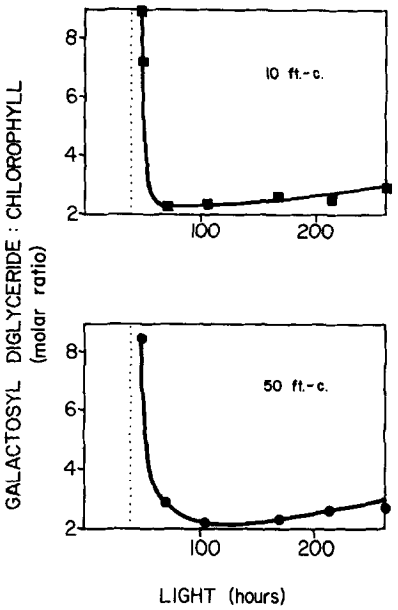

Figure 2: Molar ratio of (mono- plus di-) galactosyl diglycerides to chlorophyll in *Euglena gracilis*, strain Z, during light-induced chloroplast formation. The content of chlorophyll in the greening cells is given in Table I. The broken line parallel to the ordinate indicates the end of a latent interval which precedes the appearance of significant amounts of chlorophyll in the continuously illuminated organisms. The curve for 120-ft candles, not shown, is practically identical with that for 50 ft-candles.

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the lamellar membrane may represent a key structural event in formation of the photoreceptor apparatus in chloroplasts. It remains to be determined experimentally whether galactosyl diglyceride molecules precede chlorophyll in the lamellar membranes as foci for the insertion of chlorophyll or chlorophyll-protein complexes (8), or whether these molecules are stoichiometrically pre-assembled as packets and simultaneously inserted into the membranes of proto-chloroplastic organelles (9).

ACKNOWLEDGEMENT

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