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Short Communication

Comparative Studies of Biosynthesis of Galactolipids in *Euglena gracilis* Strain Z

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Biosynthesis of galactolipids in spinach chloroplasts has been reported (2, 5). In these studies uridine diphosphate galactose was used as a galactosyl donor to some endogenous acceptor (diglyceride) for the synthesis of galactolipids. Recently, cell-free extracts of photoauxotrophic *Euglena gracilis* were reported to catalyze the transfer of long chain acyl groups from acyl carrier protein thioester specifically to monogalactosyl diglyceride (6). It is well known that when *Euglena* cells are grown under different nutritional conditions the level of various chloroplast component varies extensively. For example, *Euglena* cells grown in the dark on heterotrophic media essentially synthesize neither galactolipids, nor sulfolipid, but when the cells were grown under photosynthetic conditions their chloroplast lipids were synthesized to the normal level (7). Similar results have been observed with other essential chloroplast components such as pigments (8) and plastoquinones (9) in *E. gracilis*. We made a comparative study on the biosynthesis of galactolipids with extracts from *Euglena* grown under different nutritional conditions: photoauxotrophic, photoheterotrophic, and strict heterotrophic conditions. The present communication deals with the results obtained in such studies.

Photosynthetic cells were obtained by growing *E. gracilis* strain Z in a 4-liter flask on an inorganic medium supplemented with vitamins B_{12} and B_1 as described by Cramer and Myers (3) and also by growing on an organic medium of Hutner (4) under the atmosphere of air–carbon dioxide (95:5%) with constant illumination by four fluorescent lamps at 25 C. Heterotrophic cultures (dark) were obtained by growing *Euglena* cells in a 1-liter flask with constant shaking under dark at 25 C. All the cells were harvested when they reached the late logarithmic phase. For photosynthetic cells it took a week to 10 days and for dark cells it was about a week before the cells reached this stage. Cells were harvested by centrifuging the cultures at 3000 rpm and washed once with 0.1 M tris buffer, pH 7.4. The washed cells were suspended in about 10 ml of the same buffer and sonified for 5 min (Branson Instruments Inc.). Chloroplasts were isolated from the broken cells by the procedure described by Carell and Kahn (F_{1b} fraction) (1). The purity of the chloroplasts was checked under a microscope. Enzymatic synthesis of galactolipids was carried out by the method described previously (2). A typical reaction mixture contained, in 1 ml: uridine diphosphate galactose (^{14}C) (0.03 μc giving 24,000 cpm with specific radioactivity of 25 μc/μmole) in 0.1 ml; chloroplasts or broken cells (dark-grown cells) equivalent to about 5 mg of proteins in 0.5 ml; 0.4 ml of 0.1 M tris buffer, pH 7.4. At the end of a 1-hr reaction at 37 C, the reaction was stopped by adding 4 ml of chloroform–methanol (2:1 v/v) and heating at 55 C for 5 min. The lipid products were extracted once more with 2 ml of the chloroform-methanol solution. The lipid extract was washed four times with 2.5 ml of 0.9% aqueous sodium chloride solution to remove residual radioactive uridine diphosphate galactose. A 1-ml aliquot of the lipid extract was transferred to a planchet, dried, and counted. The rest of the lipid extract was concentrated and separated on a column packed with silicic acid (Unisil, Clarkson Co., Williamsport, Penn.) into monogalactosyl diglyceride and digalactosyl diglyceride by eluting the column with 100 ml of a solution of 5% diethyl ether in methanol and equal volume of 20% diethyl ether in methanol, respectively. Each eluate was concentrated and counted on a planchet by means of a gas flow counter (Nuclear-Chicago). The purity of radioactive galactolipid products was determined by thin layer chromatography as described previously (10).

Table I. *Incorporation of Radioactive Galactose into Galactolipids in Euglena gracilis Chloroplasts*

Euglena Cells	Monogalactosyl Diglyceride	Digalactosyl Diglyceride	Total Lipids
	cpm/10 mg protein		
Photoauxotrophic	791	1490	3040
Photoheterotrophic	880	1240	3100
Strict heterotrophic[1]	...	...	100

[1] Broken cells were used for the enzymatic reaction.

The results shown in Table I indicate that the chloroplasts obtained from photosynthetic cells, both photoauxotrophic and photoheterotrophic, catalyze the synthesis of galactolipids, whereas dark-grown cells essentially do not have such catalytic activity. The specific activity of galactose transferase is slightly lower when broken cells instead of chloroplast preparations from photosynthetic cells were used for the enzyme assay, but such a preparation (broken cells) from heterotrophic cells essentially do not have any galactose transferase activity. It seems that photosynthetic condition is essential for the synthesis of the galactolipid-synthesizing enzyme. This result is in agreement with the observation made on the lipid composition of *E. gracilis* cells under different nutritional conditions. When *Euglena* cells were grown under photosynthetic conditions, they synthesized galactolipids in substantial quantity, whereas the cells grown under dark did not form any detectable amount of galactolipids (7). Table I also shows that the total lipid product is slightly more than the sum of mono- and digalactosyl diglyceride. This may mean that some unknown galactolipids are also produced. This aspect is under investigation. Another point is that *Euglena* chloroplasts seem to synthesize 40 to 50% more digalactosyl diglyceride than monogalactosyl diglyceride in a 1-hr reaction in contrast to spinach chloroplasts in which monogalactosyl diglyceride is al-

ways synthesized about 50% more than digalactosyl diglyceride is in the same reaction period (2, 5). In view of the differences in the kinetics of galactolipid formation in spinach chloroplasts and *Euglena* chloroplasts and especially since monogalactosyl diglyceride is reported to be converted to digalactosyl diglyceride in spinach chloroplasts (5) and this is not the case in *Euglena* chloroplasts (6), it will be quite interesting to reinvestigate the biogenesis of digalactosyl diglyceride.

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