BIOSYNTHESIS OF PHOSPHATIDYLGLYCEROL DURING GREENING OF SYNCHRONOUS DARK-GROWN *EUGLENA GRACILIS Z*

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1. Introduction

Phosphatidylglycerol is an acid phospholipid which is localized in the chloroplast membranes [1]. The configuration and chemical characterization of this phospholipid is now well established for green plants [2]. However, in isolated chloroplasts, it was not possible to show the biosynthetic pathways of phosphatidylglycerol, in particular those pathways existing in the mitochondria [3, 4].

Under these conditions it is possible to formulate the hypothesis that, during the development of chloroplast membranes, phosphatidylglycerol is derived from another phospholipid localized in a different cell compartment by a transphosphatidylation mechanism [5]. For example:

phosphatidylcholine + glycerol → phosphatidylglycerol + choline.

In order to confirm if such a mechanism could really occur in green cells, we choose dark-grown Euglena which are able to form chloroplasts in 48–72 hr when exposed to light and in the absence of any external source of carbon and nitrogen [6].

The incorporation rate of ^{32}P in the various phospholipids during one life cycle of Euglena either in the light or in the dark is reported.

Chase experiments carried out in the dark and in the light show the stability of the radioactivity incorporated in the cells.

The results indicate clearly that phosphatidylglycerol is synthesized by its own metabolic pathway.

Abbreviations:
Phospholipids: Phosphatidylglycerol = PG; Diphosphatidylglycerol = DPG; Phosphatidylethanolamine = PE; Phosphatidylcholine = PC; Phosphatidylinositol = PI; Phosphatidylserine = PS; Phosphatidic acid = AP.
After deacylation, these become, respectively: GPG, GPGPG, GPE, GPC, GPI, GPS, GP. 32-Phosphorus = ^{32}P; Glycerylphosphorylmethanol (artefact) = GPMe.

2. Materials and methods

Euglena gracilis Z is grown synchronously in the

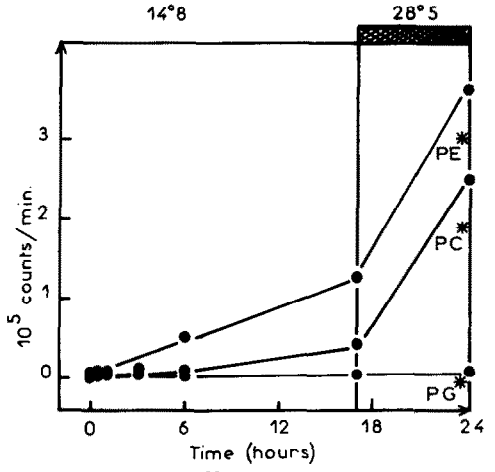

Fig. 1. Incorporation of ^{32}P during one life-cycle of *E. gracilis Z* in synchronous growth in the dark. The cell number is constant (5×10^4 cells/ml) during the cold temperature period and doubles during the warm period (10^5 cells/ml).

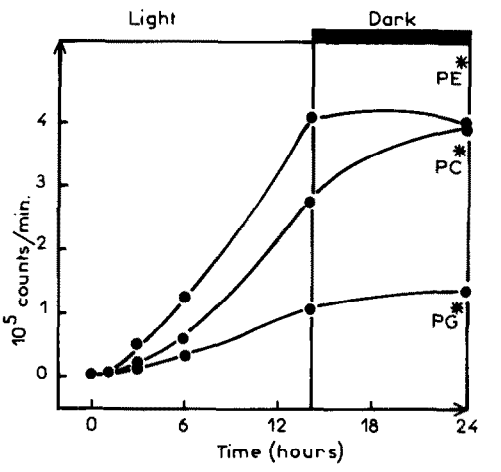

Fig. 3. Incorporation of ^{32}P during one life-cycle of *E. gracilis Z* in synchronous growth in the light. Cell number is constant during illumination time and doubles during the dark period (about 5×10^4 to 10^5 cells/ml).

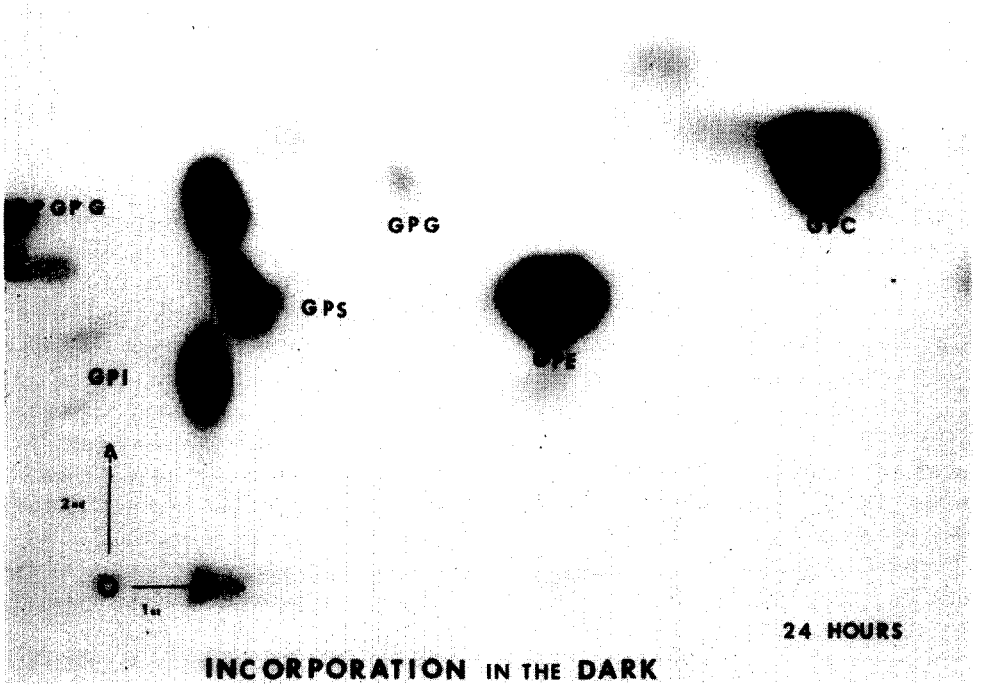

Fig. 2. Two-dimensional chromatography on Whatman paper no. 2 of phosphorus esters liberated by deacylation of the corresponding phospholipids [8] after 24 hr of incorporation in the dark. First solvent: phenol/water (100/38, v/v); second solvent: methanol/formic acid/water (80/13/7, v/v/v); revelation: autoradiography ^{32}P.

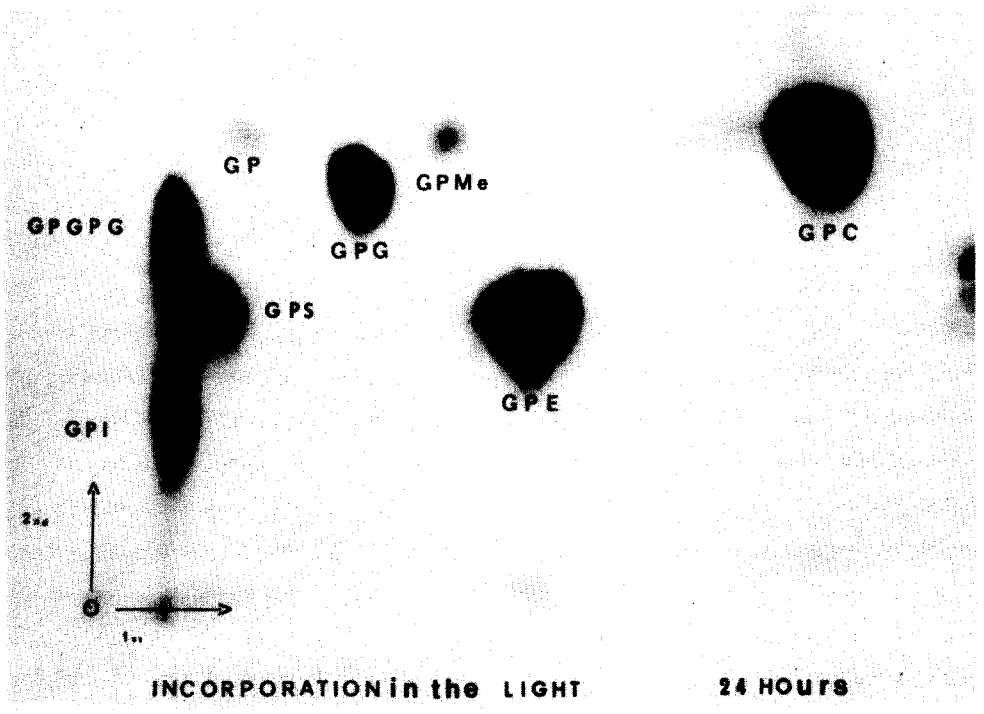

Fig. 4. Two-dimensional paper chromatography (Whatman no. 2) of phosphorus esters liberated by deacylation of *E. gracilis* phospholipids after 24 hr of incorporation in the light. First solvent: phenol/water (100/38, v/v); second solvent: methanol/formic acid/water (80/13/7, v/v/v); revelation: autoradiography ^{32}P.

dark according to the procedure described in a previous note [7].

For these experiments, the culture medium was slightly modified as follows (amounts in mg/l):
In the light:

$MgSO_4$: 200; $CaCl_2$: 20; $Fe_2(SO_4)_3 \cdot 9H_2O$: 3; $MnCl_2 \cdot 4H_2O$: 1.8; $CoSO_4$: 1.5; $ZnSO_4 \cdot 7H_2O$: 0.4; Na_2MoO_4: 0.2; $CuSO_4$: 0.02; sodium citrate: 800; vitamin B_1: 0.1; vitamin B_{12}: 0.0005; KH_2PO_4: 100; $(NH_4)_2SO_4$: 1000.

In the dark:

We added sodium acetate, 2000 mg/l.

^{32}P is incorporated into the various phospholipids during one life-cycle (24 hr) either in the dark or in the light by adding to the culture medium, just after cell division, 10 μCi/ml $Na_2H^{32}PO_4$ (C.E.A., Saclay).

For the chase experiments, the cells are washed by centrifugation 3 times with a 0.125 M phosphate buffer, pH 7, to which $MgSO_4$ is added to a final molarity of 1.25, and resuspended at the cell concentration of $0.5 - 1 \times 10^6$ cells/ml in the same buffer at 25°, either in the dark or in the light (3,000 lux) to induce the greening process.

Under these conditions, we noted very few cell divisions (less than 10%).

Cells were harvested at different times of incorporation by centrifugation at 3,000 *g*. The pellets were heated 10 min in boiling ethanol in order to inactivate the phospholipases present in Euglena.

Phospholipids were extracted, separated and identified according to a technique described previously [8] and the radioactivity was measured by gas-ionisation counting after elution of the samples. Phosphatidylglycerol was separated by thin layer chromatography on silica gel G, Merck (solvent system: chloroform:methanol:water, 65:25:4, v:v:v) eluted and the phosphorus content determined by the method of Ducet and Mencl [9]. Euglena cells were counted with a Coulter Counter model F.

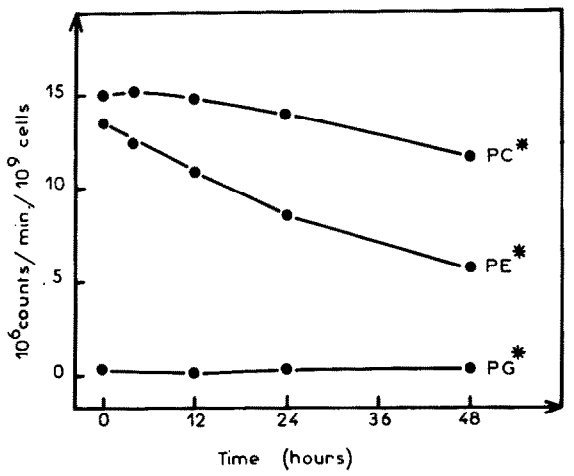

Fig. 5. Chase experiment in the dark of the radioactivity incorporated in PC and PE of *E. gracilis Z* during one life-cycle in the dark.

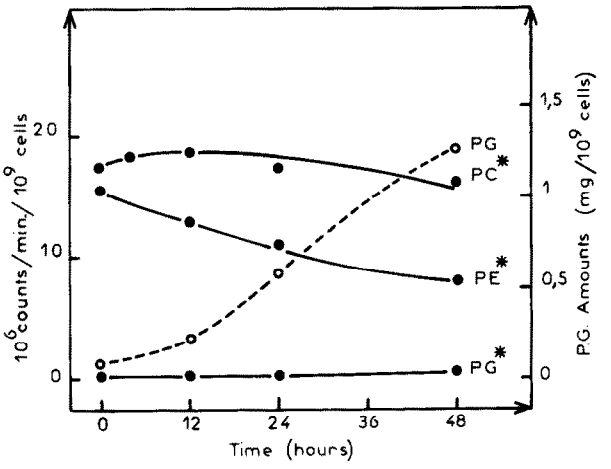

Fig. 6. Chase experiment in the light of the radioactivity incorporated in PC and PE of *E. gracilis Z* during one life-cycle in the dark (unbroken lines). Amounts of PG synthesized in mg/10^9 cells (broken lines).

3. Results and discussion

3.1. *Incorporation*

As shown in fig. 1, Euglena cells incorporated ^{32}P very intensively into their phospholipids in the dark, especially during the cell division (warm period). Phosphatidylcholine and phosphatidylethanolamine, which are present in high concentrations in the various non-plastidial membrane systems [3], are highly labelled. The same observations are made for diphosphatidylglycerol, which is selectively localized in the mitochondria [3, 10]; as can be seen in fig. 2, GPGPG (formed after deacylation of DPG) is labelled after 24 hr of incorporation in the dark.

However, phosphatidylglycerol is practically not synthesized under these conditions, as can be seen in fig. 1 and fig. 2 (the spot corresponding to GPG formed by deacylation of PG is very small). This confirms the results obtained previously [11, 12].

When the same incorporation is performed in synchronously grown green cells, phosphatidylglycerol is present in high amounts (as compared to the content found in dark-grown cells) and becomes markedly labelled (fig. 3 and 4). The most important point is that in these conditions the incorporation of ^{32}P into the phospholipids occurs during the illumination time, that means during the cell growth.

In both cases, light grown and dark grown cells, the phosphatidylethanolamine is the most rapidly synthesized phospholipid.

3.2. *Chase experiments*

3.2.1. In the dark

As shown in fig. 5, during the chase in the dark, the radioactivity incorporated in the major phospholipids (PE and PC) varies only a little (phosphatidylcholine is particularly stable). The stability of this phospholipid is understandable, since it is a membrane component and is mainly synthesized during the cell membrane formation.

However, radioactivity incorporated in phosphatidylethanolamine disappears more rapidly. This phospholipid seems to have in Euglena a much greater turnover than phosphatidylcholine. Under these experimental conditions, growth and chase, both in the dark, phosphatidylglycerol is not synthesized and therefore not labelled.

3.2.2. In the light

When the chase is performed in the light (after growth in the dark) and when the cells are then greening, the turnover of phosphatidylcholine and phosphatidylethanolamine is comparable to that shown in fig. 5 for the chase in the dark, but we

detected a large increase in the phosphatidylglycerol content (fig. 6).

However, the new phosphatidylglycerol molecules which are formed under these conditions are not labelled. But, if we incorporate ^{32}P during this greening process, phosphatidylglycerol is labelled.

Controls by electron microscopy have shown that phosphatidylglycerol synthesis follows closely the formation of chloroplast membrane systems.

Our results indicate clearly that phosphatidylglycerol, which is localized in the chloroplast lamellae, has its own biosynthetic pathway; its phosphatidyl moiety does not derive from another phospholipid by a transphosphatidylation mechanism.

Acknowledgement

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