THE POSSIBLE ROLE OF TRANSHEXADECENOIC ACID AND PHOSPHATIDYLGLYCEROL IN LIGHT REACTIONS OF PHOTOSYNTHESIS

The photochemistry and fluorescence properties of young pea leaf chloroplasts treated by phospholipase A_2

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1. Introduction

The role of the acyl lipid PG, which contains all the C16:1 trans in photosynthetic tissues is not clearly established. A close connection between its presence and grana stacking has been suggested [1] from the action of phospholipase A2 on chloroplasts from spinach leaves. A good correlation has been observed between the synthesis of C16:1 *trans* and grana stacking when etiolated corn leaves started greening under long wave monochromatic light [2]. But the correlation between C16:1 *trans* content and grana stacking is not absolute as shown on greening wheat leaves [3] and on a barley mutant [4] devoid of chl *b* and very poor in grana profiles but having a normal content in C16:1 *trans* in PG.

On the other hand, a tobacco mutant deficient in PSII and in chl LH [5] was shown to have a low content of this acid in its PG, but as it was also deficient in many other components such as chl *b*, no definitive conclusions can be drawn on the role of this lipid in thylakoids. A few years ago it was shown [6] that in spinach chloroplasts a 76% depletion in PG, induced by phospholipase A2 treatment, weakly affected the photochemical activities of photosynthesis. In this work, we show that in young pea leaves a depletion in PG, and of course in C16:1 *trans*, brings about by phospholipase A2 treatment, modifies markedly the efficiency of light collection and the rate of PQ reduction.

Abbreviations: BSA, bovine serum albumin; C16:1 *trans*, Δ3-*trans*-hexadecenoic acid; chl LH, light-harvesting chlorophyll *a*/*b* complex; PG, phosphatidylglycerol; PQ, plastoquinone; PSI, photosystem 1; PSII, photosystem 2

2. Materials and methods

Pisum sativum (cv. Annonay) plants were grown in a glasshouse at 18°C, under a 14–10 h, day–night period. Leaves from week 3 plants were ground in 50 mM MES buffer, containing 0.4 M sorbitol, 10 mM EDTA and 1% BSA (pH 6.5). The 800 × *g*, 5 min pellet was resuspended in 50 mM Hepes–NaOH buffer, 2 mM $CaCl_2$, 5 mM $MnCl_2$, 1% BSA (pH 7). After centrifugation at 100 × *g* for 5 min, the supernatant was centrifugated at 800 × *g* for 5 min and the pellet resuspended in the same medium with 0.4 M sorbitol added. Phospholipase A2 from *Vipera russelli* (1.06 Sigma unit) was then added to a suspension containing 400 μg chl (according to [7]) and incubated in the dark for 20 min at 20°C. Then the suspension was washed 4 times by centrifugation (10 000 × *g* for 1 min). All experiments were done on samples containing 6 μg chl/ml supplemented with 10^{-6} M gramicidin.

PSII activity was measured by the DCPIP-Hill reaction, with an Aminco-Chance DW2 spectrophotometer in the dual-wavelength mode (590–730 nm).

PSI activity was measured with a Clark oxygen electrode (Hansatech) by the $DCPIPH_2$–methyl vio-

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logen reaction [8].

Fluorescence induction was assayed as in [9].

Excitation energy was provided by a 250 W quartz–iodine lamp; low intensity illuminations were obtained by adding neutral density filters.

Lipids were extracted in chloroform–methanol [10]. Lipid classes were separated by TLC [11]. Methyl-esters of fatty acids were prepared by direct *trans*-esterification and analyzed by GLC [12].

3. Results and discussion

3.1. *Action of phospholipase A2 on the lipid composition of plastids*

As shown in table 1, the fatty acid composition of PG in untreated plastids was similar to the data in [13]; although the plastids were kept at 20°C for 20 min, a high content in C16:1 *trans* was observed (20%). There was no significant variations of the galactolipid content during the incubation time. These facts agree with those already published for spinach plastids [14], aged for 40 min at 20°C, in which only very small changes in PG were found.

Table 2 shows that the lipase treatment extracted >80% of PG and C16:1 *trans*. The ratio C16:1 *trans* to chl decreased from 8.1 mol/100 mol chl in untreated to 1.4 mol/100 ml chl in treated plastids. Nevertheless, in our experimental conditions, there always remained ~20% of PG in the membranes. It is obvious that the lipase depleted the thylakoid very specifically in phospholipids, mainly PG, and that on the other hand the quantity of galactolipids was not modified. These results fit well with those obtained in spinach leaves with the same incubation time [6,15]. In our experiments, the lysophospholipids resulting from the lipase treatment were below the lysophospholipids/chl ratio 0.7, known to be minimal in order to influence the photosynthetic reactions [16]. Furthermore, the addition of 1% BSA to the medium removed fatty acids and lysolipids, and prevented all the detergent effects of these compounds [17].

So, we can assert that our observations on photochemical activities are due specifically to PG and C16:1 *trans* depletion and will not result from various effects of degradative compounds released by lipase treatment or aging of the plastids.

Table 1
Fatty acid composition of phosphatidylglycerol in class II chloroplasts from young pea leaves (in % total fatty acids)

16:0	16:1 *cis*	16:1 *trans*	18:0	18:1	18:2	18:3
55.7	2.6	19.4	trace	4.7	trace	15.7

Fatty acids: 16:0 palmitic; 16:1 palmitoleic; 18:0 stearic; 18:1 oleic; 18:2 linoleic; 18:3 linolenic

Table 2
Modifications of the lipid composition of pea chloroplasts after PLA_2 treatment

	Control	Lipase
C16:1 *trans* (μg/mg chl)	23.0	4.1
$\frac{\text{mol C16:1 } trans}{\text{100 mol chl}}$	8.1	1.4
C18:3 in MGDG (μg/mg chl)	910	880
PG (μg/mg chl)	144	25
MGDG (μg/mg chl)	1510	1460

Abbreviations: PG, phosphatidylglycerol; MGDG, monogalactosyldiacylglycerol

3.2. *Photochemical activities*

We observed striking differences in photochemical activities between reference and treated plastids, unlike spinach chloroplasts studied by Hoshina and Nishida [16]. These differences were closely related to the level of excitation energy supplied (fig.1). With week 3 plants, both treated and untreated chloroplasts were saturated by >80 $W.m^{-2}$ and showed identical DCPIP-reduction rates. As the excitation energy decreased, the ratio of DCPIP-reduction rate (reference/treated) increased from 1–1.3 and then remained constant. With 1,5-diphenylcarbazide added as a substitute for the water-splitting system [18], the activity was slightly enhanced but the ratio was not modified. Similar effects were noted for PSI activity: in non-saturating conditions the ratio was ~1.3.

These experiments showed that the yield of light collection by PSI and PSII antennae was lowered by PG depletion while the activity of the photochemical

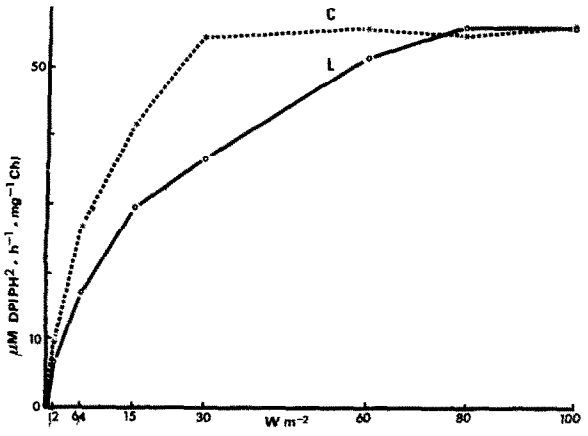

Fig.1. Saturation curve of the DCPIP-Hill reaction. C, untreated chloroplasts; L, treated chloroplasts. Chloroplast suspension 1 ml (6 µg chl) in buffer, supplemented with 25 µM DCPIP.

centers themselves was constant; moreover, PG is useful in a common part of PSI and PSII antennae such as chl LH since activities are lowered in a similar way by PG removal; however, this complex is not generally thought to transmit energy with the same efficiency to both photosystems [19] and we cannot locate the site where PG is implicated.

3.3. *Fluorescence induction*

We use Lavorel's terminology [20] for fluorescence transients. With dark-adapted chloroplasts, the shape of fluorescence induction curves was quite different in treated and untreated samples but the level of initial fluorescence rise (0 level) was the same in both cases (fig.2); consequently, there was a similar amount of 'inactive pigments' in both samples and we must correlate the loss of energy collection described above to non-radiative deactivation rather than to pigment inactivation.

The maximum level of fluorescence (P) was lower in treated than in reference plastids; the variable fluorescence was consequently reduced. In the 'I–D' phase, treated chloroplasts exhibited a faster rise to I followed by a slighter I–D dip than reference chloroplasts. It is widely accepted that the I–D dip phase is correlated with PSI activity through the plastoquinones [21] (PQ). Recently [22], it has been shown that when DCMU is added in light, the resulting fluorescence decay corresponds to the oxidized pool of PQ. In our experiments (fig.3) the variable fluorescence was decreased to 72% in the reference sample and to 80% in the treated plastids. We have come to the conclusion that ~1/3rd of the PQ pool was not reduced by PSII after lipase treatment; if this inactivation was directly related to the removal of PG by lipase, it would involve a new important role for this lipid.

When chloroplasts were preincubated in the dark with DCMU, one can investigate, during illumination, the kinetics of the primary electron acceptor reduction in PSII. In these conditions, the area between the induction curve and the asymptote, normalized

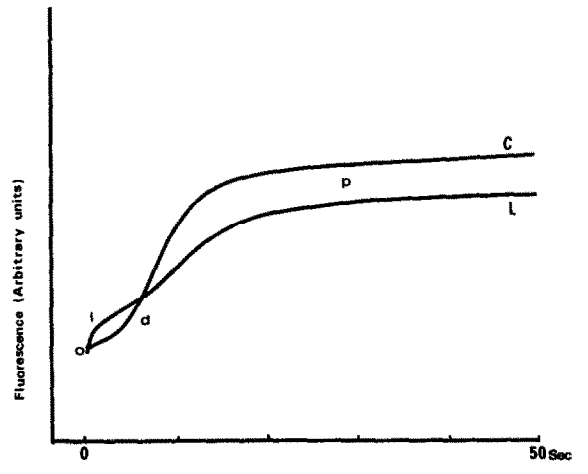

Fig.2. Kinetics of fluorescence induction: o, i, d, p, phases of induction; C, untreated chloroplasts, L, treated chloroplasts. Excitation energy, 30 W.m^{-2}.

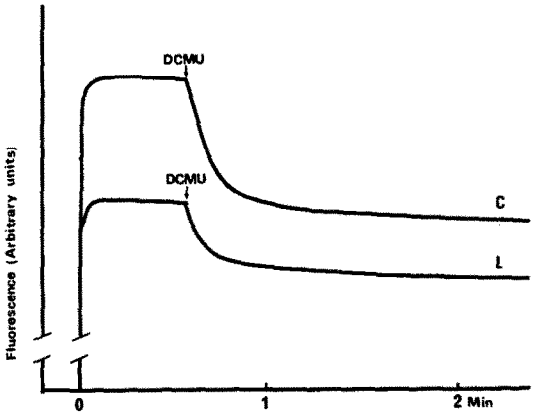

Fig.3. Kinetics of fluorescence induction; same conditions as fig.2 with 10^{-5} M DCMU added in light.

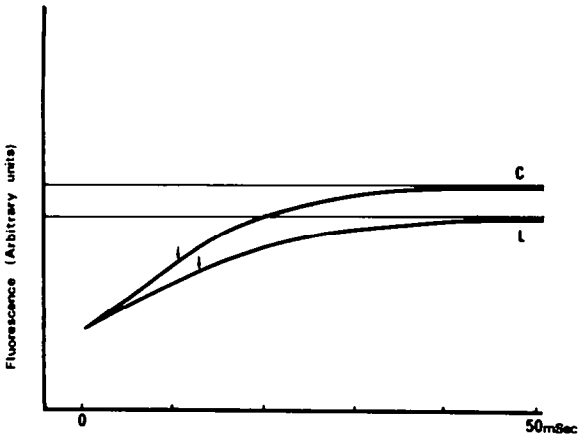

Fig.4. Kinetics of fluorescence induction, with DCMU added in the dark 10 min before measurements. Arrows show half-rise time. Same conditions as fig.2.

to maximum fluorescence level, is usually related to the amount of the primary electron acceptor of PSII [23]. We did not find (fig.4) any significant differences between the two samples which proved that they contained the same number of PSII centers.

The comparison of variable fluorescence (ratio 1.25) and half-rise time (ratio 0.85) clearly confirm that PSII antennae were less efficient in treated chloroplasts. The differences were slightly lower than those obtained by measuring the DCPIP reduction rate.

Because of these results and others on PG and C16:1 *trans*, it seems useful to recall the specific properties of this lipid and to consider its occurrence in grana stacking.

PG is a negative phospholipid. This polarity is not the most usual one among phospholipids. It contains *trans*-hexadecenoic acid which is the only fatty acid of living cell with a *trans* double bond. This bond is strangely situated at the acidic end of the molecule (polar end), all the other plant fatty acids being double bonded after the 9–10 position toward the methyl end of the molecule. From these characteristics of this phospholipid it seems to us that we may expect a specificity in its role at the membrane level.

In the thylakoid, many properties have been observed which are closely correlated to the PG level:

(i) Ageing of the membrane did not affect the PG level; this means it is a well-protected lipid inside the membrane, not easily removable by endogenous lipases which affect preferentially phosphatidylcholine; even after exogenous lipase treatment, we have shown that 20% of PG remains in the membranes.

(ii) Stacking during the greening of leaves [24] exposed to light flashes appears at the same time as the PG concentration rises, and PG containing C16:1 *trans* is located in the granal region of the plastids while PG without C16:1 *trans* is found in intergranal membranes [1].

We have to correlate these observations with the classical localization of PSII and chl LH in the granal region of plastids [25]. So we propose that some of the results reported here can be explained with a specific binding between the photosystems, chl LH, and PG (C16:1 *trans* being localized in the grana).

Certainly, PG is not only involved in the light harvesting apparatus since it also brings about depletion of the PQ-pool (activity). We must note that the PQ pool is classically shown as a network structure [26] and that the specific properties of PG may be important for the organization of this network.

All these results argue strongly for a more important role of lipids inside the membrane than that of a physical barrier as currently suggested in membrane models.

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