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REGULATION OF PHOTOSYSTEM I ELECTRON FLOW ACTIVITY BY PHOSPHATIDYLGLYCEROL IN THYLAKOID MEMBRANES AS REVEALED BY PHOSPHOLIPASE TREATMENT

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Thylakoid membranes were treated by potato lipolytic acyl hydrolase, phospholipases A_2 from pancreas and snake venom, and by phospholipase C from *Bacillus cereus* under various conditions. The changes in the uncoupled rates of electron transport through Photosystem I (PS I) and in lipid composition were followed during these treatments. Pancreatic phospholipase A_2 which destroyed all phospholipids in thylakoid membranes stimulated the $NADP^+$ reduction supported by reduced 2,6-dichlorophenolindophenol. This stimulation concerned only the dark but not the light reactions of this pathway. The main site of action of pancreatic phospholipase A_2 may be located on the donor side of PS I; the hydrolysis of phospholipids at this site caused an increased ability of reduced 2,6-dichlorophenolindophenol and ascorbate alone to feed electrons into PS I. A second site may be located on the acceptor side of PS I, probably between the primary acceptor and the ferredoxin system. When thylakoid membranes were first preincubated with or without lipolytic acyl hydrolase at 30°C (pH 8), the $NADP^+$ photoreduction was inhibited whilst the methyl viologen-mediated O_2 uptake was stimulated. A subsequent addition of pancreatic phospholipase A_2 (which had the same hydrolysis rates for phosphatidylglycerol but not for phosphatidylcholine) further stimulated the O_2 uptake and restored $NADP^+$ photoreduction. The extent of this stimulation, which depended on the presence of lipolytic acyl hydrolase, was ascribed partly to the hydrolysis of the phospholipids and partly to the generation of their lyso derivatives but not to the release of free fatty acids. On the contrary, phospholipase C which destroyed only phosphatidylcholine failed to restore this activity. It is suggested that phosphatidylglycerol is the only phospholipid associated with thylakoid membrane structures supporting PS I activities and that this lipid may play a physiological role in the regulation of these activities.

Introduction

In the thylakoid membrane, the vectorial electron transport is achieved through an adequate organization of chlorophyll-protein complexes along with proteic and nonproteic redox components [1,2]. All these constituents are distributed within a lipid environment, of which 85% are glycolipids (mono- and digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol) and 15% phospholipids (mainly phosphatidylglycerol and phosphatidylcholine).

We have previously shown that when thylakoid membranes were incubated in the presence of a nonspecific lipolytic acyl hydrolase, one could observe an inhibition of various electron-transport activities which was best explained by the hydrolysis of certain strategic lipids; the importance of these lipid molecules has been suggested to depend more upon their localization and their chemical identity than upon their relative abundance within the membrane [3]. More recently, we have studied the transmembrane

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Abbreviations: DCIP, 2,6-dichlorophenolindophenol; Tricine, *N*-tris(hydroxymethyl)methylglycine; PS, photosystem; Chl, chlorophyll.

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distribution of both phosphatidylglycerol and phosphatidylcholine in spinach thylakoid membranes as revealed by pancreatic phospholipase A_2. It was concluded that phospholipids are asymmetrically distributed across the membrane, 70% of phosphatidylglycerol and 35% of phosphatidylcholine being located in the outer leaflet and the remainder present in the inner leaflet of the membrane [4,5]. In addition, we have shown that phosphatidylcholine was the main phospholipid associated with the structures supporting PS II activity and that the phosphatidylcholine pool of the inner leaflet was essential for this activity [5]. Previous observations have indicated that pancreatic phospholipase A_2 was able to stimulate PS I electron flow activity, even under uncoupled conditions [5]. It is rather uncommon to observe an enhancement in a membrane function when part of the structures supporting this function is destroyed. Therefore, we have investigated the stimulatory effect of pancreatic phospholipase A_2 on PS I activity to determine the identity of the phospholipid(s) involved and to characterize the action site(s) of this enzyme in the thylakoid membrane.

Materials and Methods

Enzymes. Phospholipase A_2 (EC 3.1.1.4) from pig pancreas and from *Vipera russelli* venom and phospholipase C (EC 3.1.4.3) from *Bacillus cereus* were purchased from Sigma Chemical Co. and used without further purification. Lipolytic acyl hydrolase (EC 3.1.1.26) was isolated from potato tubers as described previously [3].

Biological material. Spinach (*Spinacia oleracea* var. Nobel) was grown as hydroponic culture [6]. After 14 weeks, the leaves were harvested; the thylakoid membranes were prepared as described earlier [3], suspended at a concentration of 2 mg Chl/ml and used without delay. Chlorophyll was determined as described previously [7].

Enzymatic treatments. The incubation medium for all experiments involving the lipolytic acyl hydrolase and both phospholipases A_2 was 50 mM Tricine (pH 8.0), 80 mM NaCl, 1 mM $CaCl_2$ and thylakoid membranes (0.5 mg Chl/ml). To this basic medium, lipolytic acyl hydrolase (40 μg protein/mg Chl), pancreatic phospholipase A_2 (1.6 Sigma U/mg Chl) or snake venom phospholipase A_2 (0.12 Sigma U/mg Chl) was added as indicated in the figures. For phospholipase C experiments, the incubation medium was the same as above except that 50 mM Tricine (pH 7.5) was used as buffer; phospholipase C was present at 1.4 Sigma U/mg Chl. Incubations were generally carried out in the dark at 30°C. Other conditions are specified in the legends to the figures.

Lipid analysis. The changes in the lipid content of thylakoid membranes were measured by a combination of thin-layer chromatography and scanning of the photographic negatives of the charred plates [8].

Photochemical measurements. The electron-transport activity of PS I was measured by the $NADP^+$ photoreduction (followed at 340 nm) or by the oxygen uptake in the presence of methyl viologen. The reaction mixture for $NADP^+$ photoreduction contained 50 mM Tricine (pH 8.4), 35 mM NaCl, 2 mM NH_4Cl, 4 mM sodium ascorbate, 0.3 mM DCIP, 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 2 mM $NADP^+$, 6 μM spinach ferredoxin and thylakoid membranes (100 μg Chl/ml). The oxygen uptake was measured with a Clark oxygen electrode in the same reaction mixture as above except that 2 mM NaN_3 and 0.15 mM methyl viologen were used instead of $NADP^+$ and ferredoxin; thylakoid membranes were at 20 μg Chl/ml. Other conditions are specified in the legends. The actinic light was passed through a Calflex and a wide-band DT-Red filter, both from Balzers. The irradiance was usually 250 mW/cm^2.

Results

Fig. 1 shows that when thylakoid membranes were incubated at 30°C (pH 8) in the presence of 1 mM Ca^{2+}, two uncoupled PS I electron flows (e.g., reduced DCIP to either methyl viologen/O_2 or ferredoxin/$NADP^+$) had a completely different behavior. The photoreduction of $NADP^+$ was first inhibited (approx. 50%) then progressively recovered up to the initial level. On the contrary, the methyl viologen-mediated oxygen uptake was stimulated and reached a plateau at 180%. An analysis of the lipid composition during this incubation revealed that the only change was the appearance of a small amount of free fatty acids (Table I); this could be correlated with the formation of 6-*O*-acylmonogalactosylmonoacylglycerol from 6-*O*-acylmonogalactosyldiacylglycerol (not shown in Table I but see Ref. 9). When the incuba-

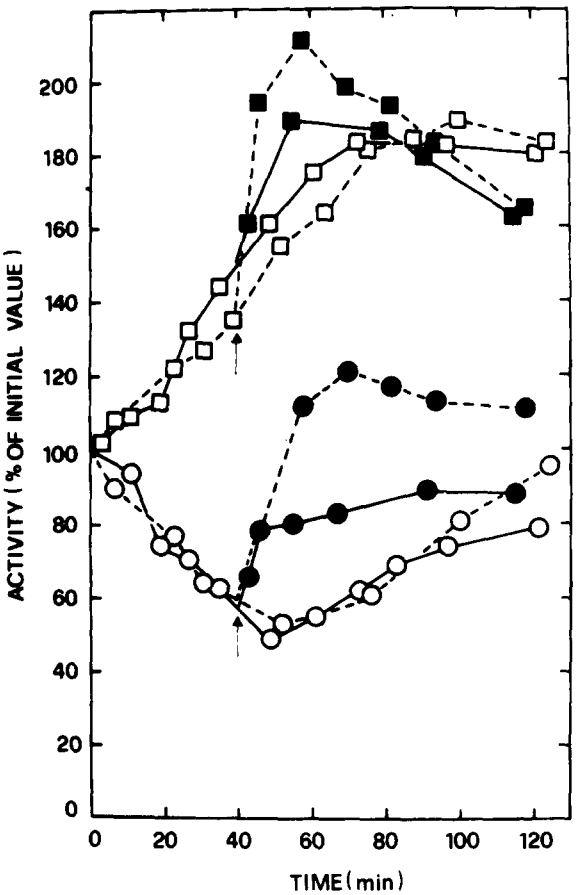

Fig. 1. Time course of the changes in the photoreduction of $NADP^+$ (○,●) and in the methyl viologen-mediated oxygen uptake (□,■) catalyzed by PS I in thylakoid membranes. The membranes were preincubated with (open symbols, full lines) and without (open symbols, dashed lines) lipolytic acyl hydrolase. After 40 min of preincubation (arrows) pancreatic phospholipase A_2 was added (closed symbols) to aliquots of both samples. The initial rates were 124 μmol O_2 consumed/mg Chl per h and 103 μmol $NADPH_2$/mg Chl per h.

tion was carried out in the presence of lipolytic acyl hydrolase, essentially the same changes in activities (Fig. 1) and in lipid composition (Table I) were observed. However, the release of free fatty acids was somewhat higher than in the control, due to an additional hydrolysis of monogalactosyldiacylglycerol.

When pancreatic phospholipase A_2 was added (after 42 min of incubation), the photoreduction of $NADP^+$ was restored rapidly and reached 110–120% of the initial activity in the controls and 80–90% in the membranes pretreated with lipolytic acyl hydrolase (Fig. 1). On the other hand, pancreatic phospholipase A_2 caused a rapid additional stimulation of the methyl viologen-mediated reaction which was also higher in the controls than in the pretreated membranes. This stimulation was followed by a decrease in these activities. In the presence of pancreatic phospholipase A_2, both phosphatidylglycerol and phosphatidylcholine were rapidly and completely hydrolyzed. In the thylakoid membranes which had not been treated with the lipolytic acyl hydrolase, the hydrolysis of phospholipids by pancreatic phospholipase A_2 was accompanied by the appearance of the corresponding lyso compounds whilst in the pre-

TABLE I

CHANGES IN LIPID COMPOSITION UNDER THE VARIOUS CONDITIONS DESCRIBED IN FIG. 1

Pancreatic phospholipase A_2 was added after 40 min of preincubation with or without lipolytic acyl hydrolase. Values expressed as nmol lipid/μmol Chl. tr, trace.

Lipid	Control				Lipolytic acyl hydrolase		
	0 min	40 min	120 min	120 min (+ phospholipase A_2)	40 min	120 min	120 min (+ phospholipase A_2)
Monogalactosyldiacylglycerol	1 431	1 316	1 260	1 260	1 280	1 121	1 068
Digalactosyldiacylglycerol	696	675	661	661	643	576	589
Phosphatidylglycerol	195	191	182	0 [a]	190	174	0
Phosphatidylcholine	150	142	141	0 [a]	134	130	0
Sulfoquinovosyldiacylglycerol	116	116	112	112	112	106	101
Free fatty acids	tr	40	98	443	87	140	830

[a] Stoichiometric amounts of lysophosphatidylcholine and lysophosphatidylglycerol present.

treated membranes no lysophospholipid could be detected due to the high activity of lipolytic acyl hydrolase towards these compounds [10]. In order to exclude the possibility of the contribution of lysophospholipids in this restoration phenomenon, we have deliberately chosen to investigate further this phenomenon under conditions where no lyso compounds can accumulate in the membrane, although the restoration was less pronounced than in the presence of lyso compounds. Since the free fatty acids released by the addition of phospholipase A_2 (see Table I) could also be part of the mechanism involved in the observed reactivation of PS I electron flow, their effect was tested under our experimental conditions. After a preincubation of thylakoid membranes in the presence of lipolytic acyl hydrolase for 40 min (see Fig. 1), a subsequent addition of linolenic acid (the most abundant free fatty acid released by the action of phospholipase A_2 and that of lipolytic acyl hydrolase on both galactolipids and 2-lysophospholipids generated by the phospholipase A_2) to these membranes (with molar ratio linolenic acid/Chl ranging from 0.1 to 20.0) failed completely to reactivate the $NADP^+$ photoreduction (data not shown). Altogether, these results indicated that the restoration of $NADP^+$ photoreduction was correlated with the hydrolysis of phospholipids but apparently not with free fatty acids per se. Furthermore, the extent of this restoration induced by phospholipase A_2 depended upon the presence of an enzyme (e.g., lipolytic acyl hydrolase) which is able to destroy the lysophospholipids generated by the phospholipase A_2.

Next, the pancreatic phospholipase A_2-induced restoration was estimated after various times of incubation with the lipolytic acyl hydrolase. Fig. 2 shows that when both enzymes were added simultaneously, the activity was first inhibited (about 40%) then decreased very slowly. In contrast, when thylakoid membranes were first incubated in the presence of lipolytic acyl hydrolase, a subsequent addition of phospholipase A_2 at different times resulted in a reactivation of the $NADP^+$ photoreduction which eventually reached the same level (about 80% of the initial rate). In addition, the longer the first incubation with the lipolytic acyl hydrolase was, the stronger was the reactivation rate (see Fig. 2). Since the reactivation was essentially due to the hydrolysis of phospholipids by the pancreatic phospholipase A_2

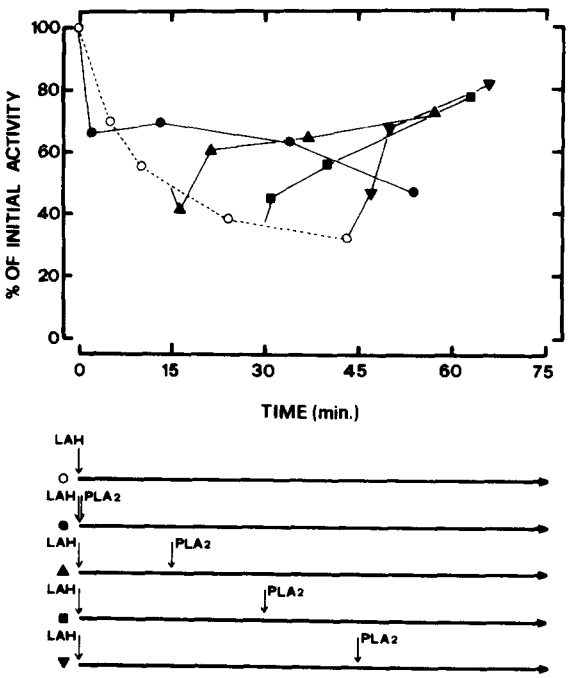

Fig. 2. Effect of pancreatic phospholipase A_2 (PLA_2) on the $NADP^+$ photoreduction catalyzed by PS I in thylakoid membranes preincubated for various times in the presence of lipolytic acyl hydrolase (LAH). (○) Treatment with lipolytic acyl hydrolase alone. The pancreatic phospholipase A_2 was added either immediately (●) or after 15 (▲), 30 (■) or 45 min (▼) of treatment with lipolytic acyl hydrolase. The initial rate was 93 μmol $NADPH_2$/mg Chl per h.

(see Fig. 1 and Table I), an experiment was designed to attempt to identify which phospholipids were involved in the restoration effect. As previously described [5], two phospholipases A_2 (from pancreas and snake venom) having opposite specificities toward phosphatidylcholine and phosphatidylglycerol were used. Fig. 3 shows that when the concentration of both enzymes was chosen so as to obtain the same hydrolysis rate of phosphatidylglycerol (and consequently different rates of phosphatidylcholine, see Fig. 5B in Ref. 5), the rate and extent of reactivation were identical. This result suggested that the rapid destruction of phosphatidylglycerol was responsible for the restoration.

A second argument which supports this suggestion is given in Fig. 4 where the abilities of pancreatic phospholipase A_2 and phospholipase C from *B. cereus* were compared. In contrast to the rapid restoration

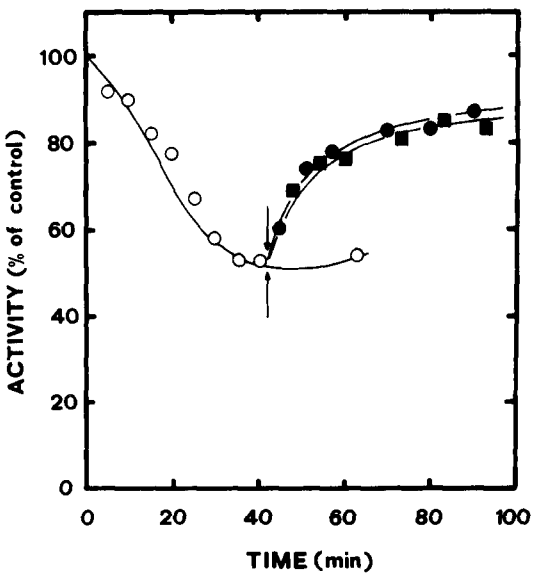

Fig. 3. Comparison between the effect of pancreatic phospholipase A_2 and that of *Vipera russelli* venom phospholipase A_2 on the $NADP^+$ photoreduction catalyzed by PS I in thylakoid membranes preincubated in the presence of lipolytic acyl hydrolase. After 42 min of treatment with lipolytic acyl hydrolase alone (○), pancreatic phospholipase A_2 (●) or snake venom phospholipase A_2 (■) was added (arrows). The initial rate was 70 μmol $NADPH_2$/mg Chl per h.

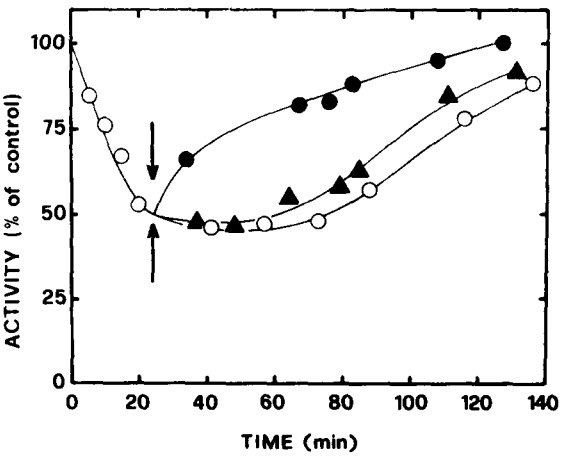

Fig. 4. Comparison between the effect of pancreatic phospholipase A_2 and that of phospholipase C from *Bacillus cereus* on the $NADP^+$ photoreduction catalyzed by PS I in thylakoid membranes preincubated in the presence of lipolytic acyl hydrolase. After 24 min of treatment with lipolytic acyl hydrolase alone (○), pancreatic phospholipase A_2 (●) or phospholipase C (▲) was added (arrows). The initial rate was 76 μmol $NADPH_2$/mg Chl per h.

induced by phospholipase A_2, the reactivation due to phospholipase C was slow and similar to that of lipolytic acyl hydrolase alone. The changes in the lipid composition occurring during this particular experiment are depicted in Fig. 5. During the treatment of the lipolytic acyl hydrolase a weak decrease in all classes of lipids (with the exception of sulfoquinovosyldiacylglycerol) was observed (see also Table I). After the addition of pancreatic phospholipase A_2, both phosphatidylcholine and phosphatidylglycerol were completely hydrolyzed. In contrast, phospholipase C destroyed completely the phosphatidylcholine (see Fig. 5, sample E_1) with a parallel production of diacylglycerol (sample E_2); this enzyme, however, attacked only slightly phosphatidylglycerol (compare samples C_2, D_2 and E_2). Again, these results suggested that the restoration of PS I electron flow was associated with the hydrolysis of phosphatidylglycerol.

The question was whether the destruction of this lipid would be related to the dark or light reactions of PS I. By investigating the relation of light intensity to the rate of the PS I electron flow reaction, one can differentiate between effects related to the dark reaction and those related to the light reaction, using the following linear equation:

$$1/V = 1/K_L \cdot 1/I + 1/K_D$$

where I is the light intensity, K_D a constant related to the dark reaction, K_L a constant related to the light reaction and V the rate of the reaction [11]. If phospholipase A_2 affects the dark reaction a change in $1/K_D$ should occur, whilst if it affects the light reaction a change in $1/K_L$ should take place. Table II shows clearly that the phospholipase A_2 treatment affected the dark reaction(s) of the $DCIPH_2$ to $NADP^+$ electron flow.

Next, we investigated whether the hydrolysis of phosphatidylglycerol would be related to the donor or acceptor side of PS I. To this end, we have measured the photoreduction of $NADP^+$ under various conditions (i.e., light intensity, concentration of electron donor or acceptor). Table III shows that 10 min incubation had essentially no effect in the controls, whatever the conditions used. On the other hand, a treatment of thylakoid membranes by phospholipase A_2 always stimulated the electron-transport rates,

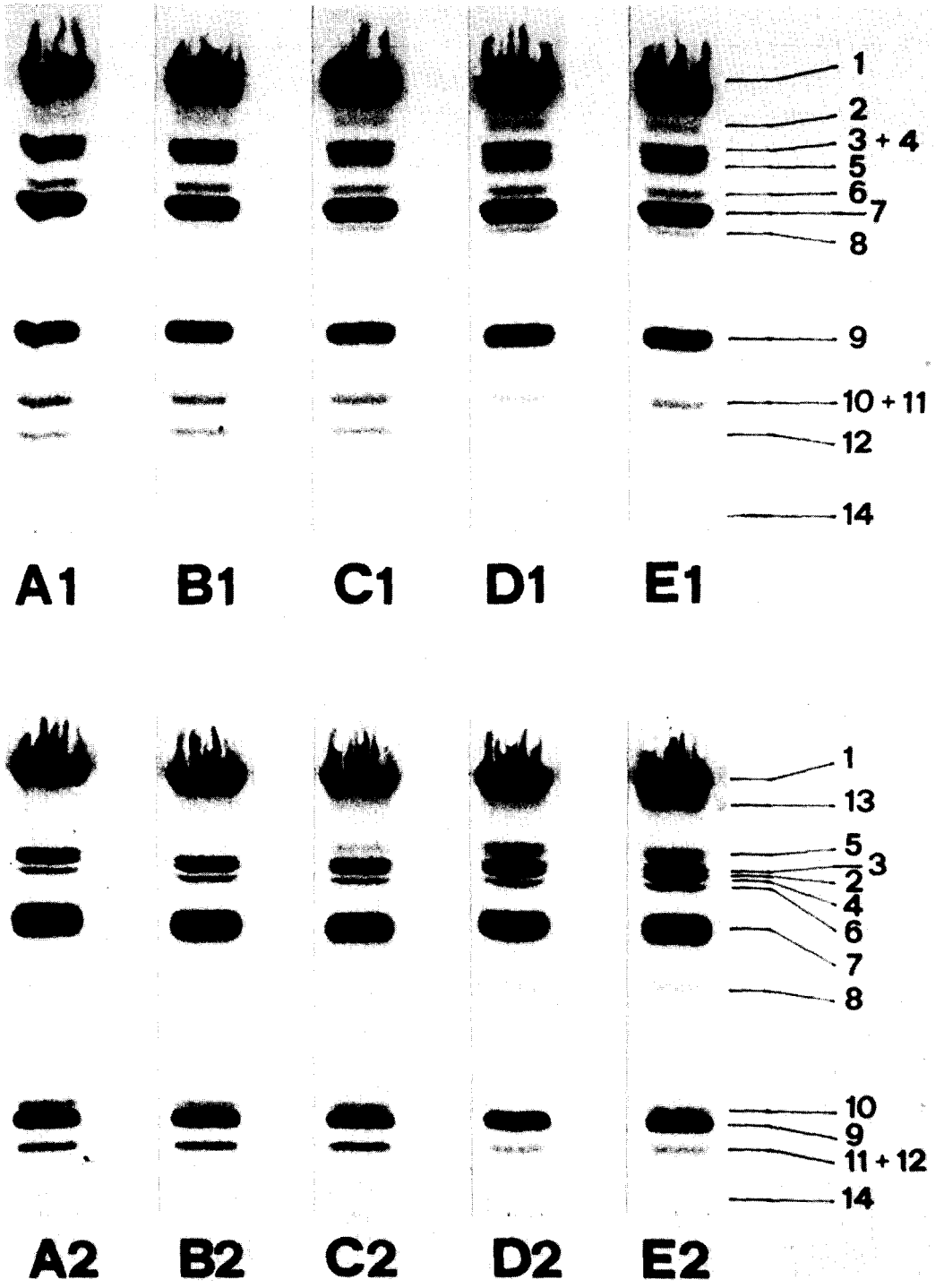

Fig. 5. Thin-layer chromatographic analysis of the lipid changes occurring during the treatment of thylakoid membranes with lipolytic acyl hydrolase alone or in combination with pancreatic phospholipase A_2 or phospholipase C from *Bacillus cereus*, as described in Fig. 4. Samples were spotted on silica gel plates and developed in $CHCl_3/CH_3OH/H_2O$ (65 : 25 : 4, v/v/v) (first row) and in $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (85 : 15 : 10 : 3, v/v/v/v) (second row). Lipids were then visualized by sulfuric acid charring [8]. Initial control (A); 24 min (B) and 130 min (C) of treatment with lipolytic acyl hydrolase alone; 130 min of lipolytic acyl hydrolase treatment with added pancreatic phospholipase A_2 (D) or with added phospholipase C (E). 1, chlorophylls + β-carotene; 2, 6-*O*-acylmonogalactosyldiacylglycerol; 3, lutein; 4, violaxanthin; 5, free fatty acids; 6, neoxanthin; 7, monogalactosyldiacylglycerol; 8, 6-*O*-acylmonogalactosylmonoacylglycerol; 9, digalactosyldiacylglycerol; 10, phosphatidylglycerol; 11, sulfoquinovosyldiacylglycerol; 12, phosphatidylcholine; 13, diacylglycerol; 14, origin.

TABLE II

CHARACTERISTICS OF THE LINES EXPRESSING THE DOUBLE-RECIPROCAL PLOTS OF RATES OF PS I ELECTRON FLOW VS. IRRADIANCE, $1/V = 1/K_L \cdot 1/I + 1/K_D$

The rates (V) were measured up to an irradiance of 290 mW/cm^2; $1/K_D$ constant related to the dark reactions) and $1/K_L$ (constant related to the light reactions) were derived from the regression analysis of the experimental data; r, correlation coefficient. After 10 min incubation at 20°C, EDTA (2 mM) was added to stop the action of pancreatic phospholipase A_2 and the rates were measured.

	$1/K_D$ ($\times 10^3$)	$1/K_L$ ($\times 10^3$)	r
Control	8.51	6.7	0.987
+ phospholipase	4.67	6.7	0.981

TABLE IV

EXTENT OF THE STIMULATION OF PS I NADP$^+$ PHOTOREDUCTION BY PANCREATIC PHOSPHOLIPASE A_2 UNDER THE SAME CONDITIONS AS DESCRIBED IN TABLE III

The extent of the stimulations was expressed as:

$$\frac{\text{Rate (phospholipase, 10 min)} - \text{rate (control, 10 min)}}{\text{Rate (control, 10 min)}} \times 100$$

Irradiance (mW/cm^2)	Electron donor		Ferredoxin	
	Type	Concentration (μM)	1 μM	10 μM
64	$DCIPH_2$	30	27	27
		300	6	16
290	$DCIPH_2$	30	58	88
		300	15	39

maximum stimulation being obtained at high irradiance and concentration of ferredoxin but at low reduced DCIP concentration. Ascorbate alone which does not support PS I electron transport in intact thylakoid membranes [12] became functional in phospholipase A_2-treated membranes under saturating conditions. The extents of the phospholipase A_2 stimulation of NADP$^+$ photoreduction are presented in Table IV. Whatever the concentrations of ferredoxin, the highest extents of stimulation were observed with limiting amounts of reduced DCIP, suggesting that the donor side of PS I is affected by the hydrolysis of phosphatidylglycerol. In addition, the extents of the stimulation were 4-times higher at low

TABLE III

DEPENDENCE OF PS I NADP$^+$ PHOTOREDUCTION ON THE IRRADIANCE, AMOUNTS OF FERREDOXIN AND ELECTRON DONORS IN THE ABSENCE AND PRESENCE OF PANCREATIC PHOSPHOLIPASE A_2

NADP$^+$ photoreduction given as uncoupled rates (2 mM NH_4Cl). At the end of each incubation period, 2 mM EDTA was added to block phospholipase A_2 activity. Incubations were at 20° C.

Irradiance (mW/cm^2)	Electron donor		NADP$^+$ photoreduction (μmol/mg Chl per h)					
	Type	Concentration	1 μM ferredoxin			10 μM ferredoxin		
			Controls		+ phospholipase (10 min)	Controls		+ phospholipase (10 min)
			0 min	10 min		0 min	10 min	
64	$DCIPH_2$	30 μM	15	15	19	40	41	52
		300 μM	14	18	19	48	49	57
290	$DCIPH_2$	30 μM	20	19	30	67	59	111
		300 μM	37	34	39	87	93	129
64	Ascorbate	1 mM	–	–	–	0	0	0
		4 mM	–	–	–	0	0	2
290	Ascorbate	1 mM	–	–	–	0	0	0
		4 mM	–	–	–	3	1	22

as compared to high concentrations of reduced DCIP with a rate-limiting amount of ferredoxin whilst they were only doubled with a saturating amount of ferredoxin. This suggests that the reducing side of PS I is also affected by the phospholipase A_2 treatment.

Discussion

It is generally believed that the destruction of certain lipids results in the impairment of the membrane functions sustained by these lipids. Adopting the enzymatic approach to make conspicuous the role of lipids in the functionality of the thylakoid membrane, several authors have shown that this was indeed the case (see Ref. 3 and references therein). In this investigation, we give evidence that under certain conditions the hydrolysis of certain membrane phospholipids gives rise to an enhancement of PS I-mediated reactions. This is rather unusual but the type and extent of the response to phospholipase A_2 were found to depend on the physiological state of the membrane at the time of the treatment.

Four cases have been considered under identical conditions of temperature (30°C) and pH (pH 8): (a) Freshly isolated thylakoid membranes were treated at zero time with phospholipase A_2; in this case phospholipids were destroyed with the formation of the corresponding lyso derivatives resulting in a stimulation of PS I activity (see Table III and Ref. 5). (b) Freshly isolated membranes were treated at zero time with a combination of phospholipase A_2 and lipolytic acyl hydrolase (Fig. 2). (c) Freshly isolated membranes were first preincubated for 40 min in the absence of lipolytic acyl hydrolase then treated with phospholipase A_2 (Fig. 1). (d) Freshly isolated membranes were first preincubated for 40 min in the presence of lipolytic acyl hydrolase then treated with phospholipase A_2 (Fig. 1).

Comparing cases a and b suggests that the stimulation of PS I activity by phospholipase A_2 can be essentially attributable to the formation of lysophospholipids. This is due to the fact that when the accumulation of lyso compounds was prevented by the presence of lipolytic acyl hydrolase, the destruction of the parent phospholipids led to an inhibition of PS I activity. Furthermore, it can be recalled that the degradation of phospholipids into their corresponding lyso derivatives and free fatty acids does not affect the bilayer structure in erythrocyte membranes [13–15]; however, it induces an increase in permeability to ions [16]. A similar situation in thylakoid membranes could explain the stimulation of the activity by an increased permeability of the membrane to electron donors. It is noteworthy that our interpretation concerning the stimulatory effect of lyso derivatives (e.g., at least in cases a and c) is in disagreement with the classical conception of the deleterious effect of lyso compounds on thylakoid membranes [17–20]. In the first phase of cases c and d, the mirror behavior of PS I activities (e.g., the stimulation of the methyl viologen-mediated reaction and the inhibition of $NADP^+$ photoreduction, see Fig. 1) was really unexpected as well as the subsequent recovery of $NADP^+$ photoreduction activity beyond 50 min of incubation at 30°C. This behavior accounts for a reversible modification of the membrane at the level of the acceptor side (i.e., from X to ferredoxin). The fact that this phenomenon did not occur at 20°C (not shown) could indicate that between 20 and 30°C a phase transition had occurred, namely in the molecular species of phosphatidylglycerol containing palmitic and 3-*trans*-hexadecenoic acids which might have changed from the gel to liquid-crystalline state. Indeed, dimyristoylphosphatidylglycerol has been shown by differential scanning calorimetry to undergo a sharp transition at about 25°C [21].

In the second phase of case d, the restoration of the activities can be explained by the hydrolysis of phospholipids. The additional restoration of the activity in case c seems to be due mainly to the presence of lysophospholipids and possibly to some galactolipids which have not been destroyed by lipolytic acyl hydrolase.

It is noteworthy that at the beginning of the preincubation in the presence of lipolytic acyl hydrolase, certain phospholipids are required (case b) whilst during the preincubation they must be destroyed (case d) for full photochemical activity (see Fig. 2). This switch in the role of certain phospholipids may be an expression of a lateral redistribution of the membrane components.

One can also ask whether the free fatty acids per se released by these enzymes could be involved in the mechanism(s) of the reactivation. The effects of added free fatty acids on photosynthetic electron transports depend on their concentration and on the

pH of the reaction medium [19,22–24]. At pH values higher than 7.5, these effects are stimulatory at low fatty acid/Chl ratios and inhibitory at higher ratios. Under our experimental conditions, an addition of linolenic acid instead of phospholipase A_2 (see Fig. 1) did not reactivate the PS I $NADP^+$ photoreduction. However, it is not correct, sensu stricto, to compare the effects of an addition of lipid on an intact membrane with those of an equal amount of lipid released endogenously (see, for instance, Refs. 9 and 13). In these two cases, a threshold fatty acid/Chl ratio might exist above which an inhibition of electron transport occurs. The extent of the reactivation observed in the presence of a relatively small amount of free fatty acids (443 nmol/μmol Chl, case c) was much greater than that obtained in the presence of twice this amount (830 nmol/μmol Chl, case d; cf. Fig. 1 and Table I). If these endogenously released free fatty acids had been actively involved in the reactivation, then the opposite should have been observed. Finally, one may suggest that the type of free fatty acid released but not its concentration could be responsible for the reactivation. In this case, such a free fatty acid could have been released only from the 2 position of certain phospholipids by the action of the phospholipases A_2. Obviously, the levels of this hypothetical, particular free fatty acid would be equal in cases c and d, since the pancreatic phospholipase A_2 was able, in both cases, to destroy all phospholipids of the thylakoid membrane (Table I). As a consequence, the extent of reactivations should be the same in cases c and d, which is obviously not the case (Fig. 1).

Previous studies presented indirect evidence that phospholipids were associated with PS I structures in the thylakoid membrane [25–28]. Here, phosphatidylglycerol has been identified as the only phospholipid the destruction of which was responsible for both stimulation (case a) and restoration (cases c and d) of PS I activity. This has been indirectly verified by the experiments comparing the effect of phospholipases A_2 from snake venom and pancreas (Fig. 3) and demonstrated by comparing the effect of phospholipases A_2 and C (Figs. 4 and 5). These results exclude the possibility of the involvement of phosphatidylcholine in the structures which participate in the restoration of PS I activity. This conclusion supports our previous results [5] but disagrees with other findings [29–31].

Concerning the type of reactions which are affected by the hydrolysis of phosphatidylglycerol, the results showing the stimulatory action of phospholipase A_2 alone on PS I electron flow (case a) permit us to exclude the possibility of the effect of this enzyme on the light reactions (Table II). Two types of dark reactions can be concerned, e.g., those on the oxidizing and those on the reducing sides of PS I. To discriminate between these two possibilities, the following rationale has been adopted. Under conditions where the irradiance and ferredoxin were not rate limiting, an effect of phospholipase A_2 on the donor side should be stronger at low than at high concentration of the electron donor. This was indeed the case, suggesting that the hydrolysis of phosphatidylglycerol resulted in increased permeability of the membrane to reduced DCIP. This was further substantiated by the appearance of a PS I activity supported by ascorbate alone (Table III). Such a phenomenon was already observed when thylakoid membranes were treated with lipolytic acyl hydrolase [3] although in this latter case the ascorbate-supported activity was suppressed by a longer incubation period. On the other hand, this activity was permanent with phospholipase A_2 treatment, suggesting the involvement of lysophospholipids in this process.

The donor side thus appears to be the main target of pancreatic phospholipase A_2 in the PS I region. However, the extent of the stimulation can be modulated by the action of the enzyme on a second target located at the acceptor side. This modulation is expressed by the fact that the stimulation at a low as compared to a high concentration of reduced DCIP was 2-times higher at limiting than at saturating ferredoxin concentration (Table IV).

It has been suggested that the phosphatidylglycerol molecules, namely those containing 3-*trans*-hexadecenoic acid, could exert a cementing effect in the building of appressed regions in thylakoid membranes [32]. However, this hypothesis has been challenged [33]. Although the aim of this study was not to solve this problem, we can now suggest another possible physiological role of phosphatidylglycerol as an element which may regulate the rate of PS I electron transport.

In conclusion, from these results and those recently published [5], it may be suggested that in addition to their asymmetric transmembrane distribu-

tion [5], the phospholipids are located in two main, spatially distinct pools within the plane of the membrane, phosphatidylcholine being associated with PS II [5] and phosphatidylglycerol with PS I structures.

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