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Involvement of distinct populations of phosphatidylglycerol and phosphatidylcholine molecules in photosynthetic electron-flow activities

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When spinach thylakoid membranes were treated with pancreatic phospholipase A_2, phospholipids were degraded and the uncoupled non-cyclic electron-flow activity (from H_2O to $NADP^+$) was progressively inhibited. To discriminate between the relative contributions of the hydrolysis products (free fatty acids and lysophospholipids) and of the phospholipid depletion per se to inhibit the activity, we made use of the known property of bovine serum albumin to remove such hydrolysis products from membranes. Using careful washings and adequate lipid extraction procedures, we could ascertain that all hydrolysis products generated by phospholipase A_2 were effectively removed from the thylakoid membrane by bovine serum albumin treatment. When bovine serum albumin was added to thylakoid membranes after various incubation times with the phospholipase A_2, the electron-flow activity was rapidly, but not completely restored. However, when phospholipid hydrolysis exceeded a certain extent (70–85%), the activity was totally inhibited and its restoration by albumin was no longer possible. Addition of EGTA to the phospholipase A_2-treated membranes blocked both the enzyme action and the progress of electron-flow inhibition. Under these conditions, the amplitude of the albumin-induced restoration of electron-flow rate did not depend on the time span between EGTA block and albumin addition. We show that phospholipid depletion of thylakoid membranes is entirely responsible for the irreversible (albumin-insensitive) inhibition of the electron flow from H_2O to $NADP^+$ by phospholipase A_2. Plotting the extent (%) of this inhibition vs. the extent (%) of phospholipid depletion allowed us to distinguish three populations of both phosphatidylglycerol and phosphatidylcholine. The first one, which was easily accessible to the enzyme, did not support greatly the electron-flow activity (around 40% of each phospholipid destroyed vs. only 10% or less inhibition). On the other hand, the electron-flow activity strongly depended on the second, less accessible population of phospholipids (around 40% of each phospholipid destroyed vs. 90% inhibition). Finally, the third population of phospholipids was not involved in the uncoupled non-cyclic electron flow activity.

Abbreviatons: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetate; disodium salt), PC, phosphatidylcholine; PG, phosphatidylglycerol; Mes, 4-morpholineethanesulphonic acid; Mops, 4-morpholinepropanesulphonic acid; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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Introduction

Electron transport in thylakoid membranes is achieved through an adequate spatial organization of chlorophyll-protein complexes linked together by protein and non-protein redox components [1]. All these constituents are distributed within a lipid environment which is no longer considered as a simple, homogenous bilayer, but characterized by

transversal [2–4] and lateral [5–7] heterogeneity. Thus any alterations in the lipid membrane environment may alter the conformation, the orientation and, eventually, the function of proteins involved in the photosynthetic electron flow. One of the purposes of this investigation is to obtain evidence that thylakoid membrane phospholipids are required for efficient photosynthesis. However, the functional role of lipids in a membrane is an ambiguous concept because lipids, in contrast to most proteins, have by themselves no recognized catalytic properties. Thus, it is extremely difficult to dissociate the functional from the structural role of lipids. So far, most of the approaches adopted to determine the involvement of lipids in the function of photosynthetic membranes have been aimed at associating the occurrence, the composition, the modification or the depletion of certain lipids with a particular function in thylakoids [8–11].

One of the best methods to tackle this problem appears to be the enzymatic approach which consists of treating thylakoid membranes with specific lipolytic enzymes under various conditions (temperature, ionic strength, etc.) and of measuring simultaneously lipid hydrolysis and the changes occurring in several types of electron-transport activity. The prerequisites, the advantages and drawbacks of this approach have been discussed recently [3,8]. Although this method has given quite valuable information on the role of lipids in thylakoid membrane function (Ref. 11 and references therein), most of the results are tarnished by the fact that hydrolysis products resulting from the enzymatic reaction may by themselves interact with the photosynthetic components of the membrane. Indeed, it is known that exogenous [12–14] and endogenous [14–16] free fatty acids as well as exogenous lysophospholipids [17] can impair considerably the structure and function of thylakoids. Thus, it is often impossible to make a distinction between the effect of the parent lipid depletion itself and of the hydrolysis products themselves. In order to avoid such an ambiguity in the interpretation of the results, we have treated the thylakoid membrane with pancreatic phospholipase A_2 and made a systematic use of bovine serum albumin which is known to remove most free fatty acids and lysophospholipids from membranes [18]. In this way, any change in electron-transport activity following phospholipase A_2 treatment can be attributed unequivocally either to the decrease in the amount of native phospholipids or to the hydrolysis products which are released in the membrane.

In this investigation, we show unambiguously that the phospholipid depletion, but not the release of hydrolysis products, is responsible for the irreversible inhibition of electron flow activities. Moreover, we show under which conditions such a correlation can be expected.

Materials and Methods

Enzymes and chemicals. Phospholipase A_2 from porcine pancreas was purchased from Sigma Chemical Co., or Boehringer and used without further purification. Before each experiment, aliquots of phospholipase A_2 suspension were diluted with water.

Preparation of defatted bovine serum albumin (adapted from Ref. 19). Bovine serum albumin was purchased from Fluka A.G. Albumin (10 g) was washed as follows: (a) in 100 ml acetone under stirring for 15 min followed by filtration; (b) step (a) was repeated; (c) in 100 ml ethanol followed by filtration then rinsed with acetone and vacuum dried. This complete cycle was repeated two times. Only under these conditions was an extract in acidified hexane/isopropanol (3 : 2, v/v) devoid of (lyso)phospholipids.

Preparation of thylakoid membranes. Spinacia oleracea (var. Nobel) was grown in a growth chamber and the leaves were harvested after 14 weeks of hydroponic culture [20]. Intact chloroplasts were prepared according to Ref. 21 in a medium containing 330 mM sorbitol, 20 mM Mes/0.2 mM $MgCl_2$/ajusted to pH 6.5 with 1 M Tris. Intact chloroplasts were shocked osmotically in H_2O for 15 s, then an equal volume containing 2 mM Mops/200 mM sorbitol/2 mM KOH was added and the final medium adjusted to pH 7.5 or 8.0. After centrifugation for 3 min at 4300 × *g*, the resulting pellet was resuspended in the above medium. Thylakoids were washed twice, resedimented and resuspended in the last medium supplemented with 150 mM NaCl to a concentration of 2 mg chlorophyll/ml.

Enzymatic treatments. The incubation of thylakoid membranes (0.5 mg chlorophyll/ml) with phospholipase A_2 was carried out in darkness at 20°C in 50 mM Tricine (pH 8), 35 mM NaCl, 1 mM $CaCl_2$, phospholipase A_2 (1.6–5 unit/mg chlorophyll) and, where indicated, 20 mg bovine serum albumin/ml. Aliquots of the above medium were washed twice with a bovine serum albumin-free medium (50 mM Tricine (pH 8)/35 mM NaCl), taken at various times (including a zero time control without phospholipase A_2), for the determination of electron-transport activities and lipid analysis. This extensive washing procedure was necessary to remove completely any bovine serum albumin loaded with hydrolysis products and which sticked to the membrane. The reactions catalyzed by the phospholipase, as well as the controls were stopped by the addition of EGTA (1–4 mM EGTA adjusted to pH 8) at 2°C.

Determination of electron-flow activities. Uncoupled non-cyclic electron flow activity (Photosystem II plus I) was measured at 20°C by the photoreduction of $NADP^+$ (340 nm) from H_2O in a reaction mixture containing 50 mM Tricine (pH 8.4)/35 mM NaCl/2 mM $NADP^+$/70 μg ferredoxin per ml/2 mM NH_4Cl/thylakoids (100 μg chlorophyll/ml). Photosystem II electron flow was determined by the photoreduction of DCIP (at 589 nm) at 20°C in a reaction mixture containing 50 mM Mops (pH 7.6)/35 mM NaCl/0.2 mM DCIP/0.01 mM dibromothymoquinone/2 mM NH_4Cl/thylakoids (40 μg chlorophyll/ml). Photosystem I electron flow was measured by the consumption of O_2 (Clark-type electrode) in 50 mM Tricine (pH 7.8)/35 mM NaCl/0.15 mM methyl viologen/2 mM NaN_3/2 mM NH_4Cl/0.01 mM DCMU/4 mM sodium ascorbate + 0.1 mM DCIP/thylakoids (20 μg chlorophyll/ml). The intensity of actinic light after filtration through a Calflex and DT-red filter was about 29 $mW \cdot cm^{-2}$ at the level of the cuvette (2 mm pathway) and was found to be saturating for the electron-transport activity.

Lipid analysis. Two lipid-extraction procedures were used. When only diacylphospholipids were determined (Table I and Fig. 4) thylakoid suspensions (560 μl) were extracted by 1 ml chloroform/methanol (53:37) resulting in a two-phase system [22]. When lysophospholipids remaining in thylakoid membranes were determined, thylakoid pellets were extracted in a one-phase system (chloroform/methanol; 1:3) to avoid loss of lysophospholipids commonly encountered in a two-phase system [23]. After the separation of lipid classes on silicagel-60-coated plates developed in acetone/benzene/water (91:30:8), the relative phospholipid composition of control and phospholipase-treated thylakoid membranes was routinely determined according to Ref. 24. For lysophospholipid separation, a mixture of acetone/toluene/methanol/water (90:25:7:12) was used. In experiments reported in Table II, free fatty acids were separated from the other lipids and pigments by TLC with acetone/petroleum ether (b.p. 40–60°)/acetic acid (10:9:1), scraped then methylated and analyzed by gas chromatography with docosanoic acid as internal standard.

Results and Discussion

We have already shown that, as a consequence of phospholipid hydrolysis in thylakoid membranes treated by pancreatic phospholipase A_2, the uncoupled activities of both non-cyclic electron flow ($H_2O/NADP^+$) and of Photosystem II alone (H_2O/DCIP in the presence of dibromothymoquinone) rapidly decrease and eventually are completely inhibited [2,25]. In addition, during these incubations with phospholipase A_2, the content of the three membrane glycolipids (mono- and digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol) remains essentially constant [25]; this is due to the alkaline condition employed (pH 8) which inhibits most of the endogenous lipolytic and transacylation activities commonly encountered in spinach thylakoids incubated at pH < 7.5 [14,26]. Therefore, the primary cause of the phospholipase A_2-induced inhibition of electron-flow activity must be ascribed to the decrease in the amount of native phospholipid molecules and/or to the release of hydrolytic products (lysophospholipids and free fatty acids) in the membrane. We can thus consider that both phospholipid depletion and phospholipid hydrolysis products will separately contribute to the phoshpolipase A_2-induced inhibition of activities. In the experiments illustrated in Fig. 1, we measured the uncoupled non-cyclic electron-flow activity in

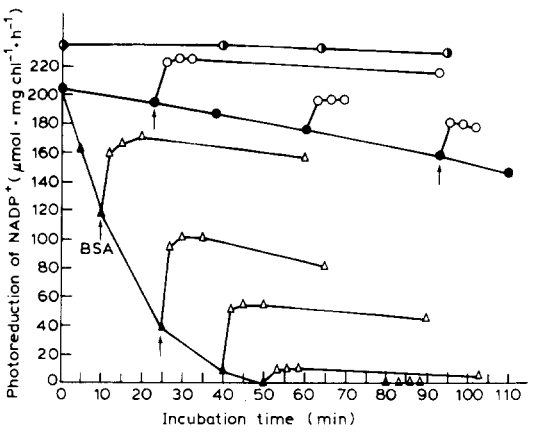

Fig. 1. Time-course of $NADP^+$ photoreduction rates (from H_2O) in the presence of phospholipase A_2 (4 Sigma units/mg chlorophyll) and bovine serum albumin (20 mg/ml) added after various hydrolysis times (see arrows); ○, controls with bovine serum albumin; ●, controls without bovine serum albumin; ▲, phospholipase A_2-treated samples; △, phospholipase A_2-treated samples followed by a further addition of bovine serum albumin at the times indicated (see arrows). After addition of bovine serum albumin, no free fatty acids and lysoderivatives remained in the thylakoid membrane (see Tables II and III). BSA, bovine serum albumin.

control and phospholipase A_2-treated thylakoid membranes. Sufficient bovine serum albumin was added after various incubation times in order to remove hydrolysis products from membranes completely. A slight stimulation of the activity was observed when control membranes were supplemented with bovine serum albumin, regardless of its addition time. Generally, control membranes incubated in the presence of bovine serum albumin exhibited a remarkable stability of their electron flow activity. On the other hand, phospholipase A_2 induced a rapid inhibition, which was eventually complete. However, addition of bovine serum albumin caused a rapid restoration of electron-transport rates. This restoration was characterized by the fact that (a) it did not reach the corresponding control level (except for very short incubation times) and (b) its amplitude was constant over 30 min incubation, but decreased rapidly upon further incubation with phospholipase A_2, so that after 80 min, bovine serum albumin failed completely to restore the activity. We conclude that as a consequence of the removal of hydrolysis products by bovine serum albumin, part of the total inhibition induced by phospholipase A_2 can be reversed, as long as phospholipid depletion did not exceed a certain degree (from 40 min incubation, see Fig. 1 and Table I).

The albumin-insensitive part of the inhibition of activity, which also increased during incubation time (Fig. 1), must therefore be explained by other reasons. Two possibilities may be considered: the irreversible (bovine serum albumin-insensitive) inhibition may be due to (a) a dose effect of the hydrolysis products and/or (b) phospholipid depletion. One would expect explanation (a) to be true if it would be possible to show that a given amount of hydrolysis products would exert a time-dependent inhibitory action. Fig. 2 shows how we can decide between these two possibilities. Thylakoid membranes were treated with phospholipase A_2, but at various times (arrows), an excess of the calcium chelator EGTA was added to aliquots of the initial suspension, thus, blocking immediately the hydrolysis of phospholipids (results not shown). Five populations of membranes were thus obtained (Fig. 2), in which the extent of phospholipid degradation was regularly increased (Table I). These five populations were further incubated for extended periods, each successive population being characterized by a higher amount of hydrolytic products which did not change over the remaining incubation time. These hydrolytic

TABLE I

PHOSPHOLIPID CONTENT OF THYLAKOID MEMBRANES TREATED BY PANCREATIC PHOSPHOLIPASE A_2

Data refer to those of Fig. 1. Conditions as in Fig. 1. The 100% values corresponded to 42.4 and to 152.9 nmol per mg chlorophyll for phosphatidylcholine and phosphatidylglycerol, respectively.

Incubation time with PLA_2 (min)	Phosphatidylglycerol (% lipid class)	Phosphatidylcholine (% lipid class)
0	100	100
3	57	57
20	45	43
50	30	14
80	22	8

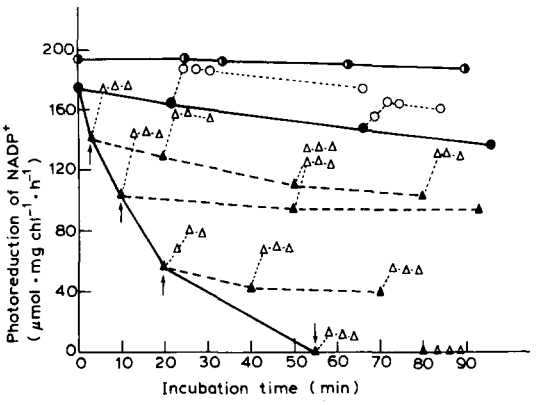

Fig. 2. Time-course of $NADP^+$ photoreduction rates (from H_2O) in the presence of phospholipase A_2 (4 Sigma units/mg chlorophyll). Bovine serum albumin (20 mg/ml) and EGTA (4 mM) added after various hydrolysis times (see arrows). O, controls with bovine serum albumin; ●, controls without bovine serum albumin; ▲, phospholipase A_2-treated samples. Aliquots of phospholipase A_2-treated samples were then treated (see arrows) by EGTA (4 mM) (▲- - - - -▲) and further supplemented with bovine serum albumin (△·····△) after various times.

products were then removed by adding bovine serum albumin either immediately after EGTA addition or after various incubation times. During this experiment, the uncoupled non-cyclic electron-flow activity ($H_2O/NADP^+$) was measured (Fig. 2). The addition of EGTA to phospholipase A_2-treated membranes drastically stopped the inhibition of activity. Moreover, the residual electron-transport activity of these EGTA-treated membranes was remarkably stable over the time scale of the experiment. In addition, the amplitude of the albumin-induced restoration did not depend on the time span between EGTA and bovine serum albumin additions. Altogether, these results show that any time-dependent, dose effect of hydrolysis products can be definitely excluded as a possible explanation for the irreversible inhibition of electron flow activity. Therefore, the only explanation for this inhibition should be the phospholipid depletion in thylakoid membranes.

In order for this conclusion to be true, it was crucial to demonstrate that, under our conditions, the addition of bovine serum albumin removes completely the hydrolysis products from the thylakoid membrane. However, it is worth mentioning that aging of thylakoid membranes results in a slow, time-dependent release of free fatty acids which arises essentially from galactolipids [27]. Therefore, in phospholipase A_2-treated membranes, the amount of free fatty acids measured will be due to the contribution of both the enzymatic hydrolysis itself and of the aging process. In addition, freshly prepared thylakoids already contained a low level of endogeneous free fatty acids (42 nmol per mg chlorophyll, see Table II),

TABLE II

REMOVAL BY BOVINE SERUM ALBUMIN OF FREE FATTY ACIDS GENERATED IN THYLAKOID MEMBRANES TREATED BY PHOSPHOLIPASE A_2

Experimental conditions were those of Fig. 2. Total FFA (free fatty acids) corresponded to the sum of endogenous and phospholipase A_2 (PLA_2)-released FFA. Endogenous FFA level in PLA_2-treated samples was assumed to be similar to the corresponding controls. PLA_2-released FFA were calculated from the hydrolysis extent (from 0 to 80 min) of parent phospholipids, and their actual level in the thylakoid membrane was determined by substracting the endogenous FFA level from the total amount of FFA after 80 min of PLA_2 treatment. When bovine serum albumin (BSA) was added in the reaction mixture, samples were taken up 8 min later for lipid analysis.

Experimental conditions	(nmol per mg chlorophyll)					
	PC	PG	total FFA (measured)	endogenous FFA level	PLA_2-released FFA	
					calculated	measured
Control, 0 min	44	144	42.0	42.0	0	0
Control, 80 min	36.7	140.4	139.8	139.8	0	0
Control, 0 min + BSA	46.8	136.8	42.4	42.4	0	0
Control, 80 min + BSA	37.4	141.8	51.8	51.8	0	0
PLA_2, 80 min	0	8.6	317.0	139.8	179.4	177.2
PLA_2, 80 min + BSA	0	4.2	51.0	51.8	179.4	0

which corresponds to 2.7% of total acyl lipids. Since these free fatty acids did not contain any *trans*-3-hexadecenoic acid which is typical of phosphatidylglycerol (not shown), one can safely conclude that this increase in endogenous free fatty-acid level arises from glycolipids. In all the control samples (0 and 80 min) the addition of bovine serum albumin changed neither the phospholipid content of thylakoid membranes nor the level of free fatty acids encountered in freshly prepared thylakoids. This suggests that during aging most free fatty acids are released in the outer monolayer and thus are easily removable by bovine serum albumin. It must be emphasized that under all these control conditions (± albumin, 0 and 80 min) the uncoupled electron flow remained very active (see Fig. 2). Keeping in mind that a low endogeneous, albumin non-removable level of free fatty acids is present in all thylakoid samples, Table II shows clearly that phospholipase A_2-released free fatty acids were completely removed by bovine serum albumin. In addition the free *trans*-3-hexadecenoic acid was found in phospholipase A_2-treated samples, only when bovine serum albumin was absent. Thus, we conclude that the inhibition of electron flow activity by phospholipase A_2 cannot be ascribed to the release of free fatty acids by this enzyme.

A similar approach was adopted to determine whether those lysophospholipids generated in thylakoid membranes by the action of phospholipase A_2 were efficiently removed by bovine serum albumin washings. In these experiments (Table III), lipids were extracted using a one-phase system to ensure a complete recovery of lysophospholipids remaining in membranes. The results of Table III demonstrate in a straightforward way that: (a) low levels of endogenous lysophospholipids were detectable in control membranes; (b) these endogenous lysophospholipids were innocuous with respect to electron-flow activity (compare Table III and Fig. 2); (c) the washing procedure decreased the recovery of lysophosphatidylglycerol, whereas it did not affect that of lysophosphatidylcholine; (d) the high amounts of lysophospholipids generated by phospholipase A_2 in thylakoid membranes were completely removed by our washing procedure with bovine serum albumin. We conclude therefore that the irreversible inhibition of non-cyclic electron flow by phospholipase A_2 treatment (Figs. 1 and 2) is entirely attributable to the phospholipid depletion of thylakoid membranes and that hydrolysis products (lysophospholipids and free fatty acids) do not play any significant role in this respect.

The comparison of Figs. 1 and 2 shows that the

TABLE III

REMOVAL BY BOVINE SERUM ALBUMIN OF LYSOPHOSPHOLIPIDS GENERATED IN THYLAKOID MEMBRANES TREATED BY PHOSPHOLIPASE A_2

Experimental conditions were those of Fig. 2. Total lysophosphatidylcholine (LPC) and lysophosphatidylglycerol (LPG) corresponded to the sum of endogenous and phospholipase A_2 (PLA_2)-released lysophospholipids. Endogenous LPC and LPG levels in PLA_2-treated samples were assumed to be similar to the corresponding controls. PLA_2-released level of LPC and LPG correspond to the amount of lysophospholipids effectively measured in thylakoid membranes minus the amount of these compounds in the corresponding controls. When bovine serum albumin (BSA) was added in the reaction mixture, samples were taken up 8 min later for lipid analysis.

Experimental conditions	(nmol per mg chlorophyll)							
	PC	PG	total		endogenous level of		PLA_2-released level of	
			LPC	LPG	LPC	LPG	LPC	LPG
Control, 0 min	40.7	161.7	0	2.2	0	2.2	0	0
Control, 80 min	39.6	156.6	1.5	2.3	1.5	2.3	0	0
Control, 80 min + BSA	38.5	160.8	3.7	7.1	3.7	7.1	0	0
PLA_2, 80 min	5.2	12.4	32.8	90.8	1.5	2.3	31.3	88.5
PLA_2, 80 min + BSA	1.0	15.2	3.9	3.7	7.1	0	0.2	0

amplitudes of bovine serum albumin-dependent activities were smaller when EGTA was added to phospholipase A_2-treated membranes. Fig. 3 shows that this can be attributed to the loss of endogenous Ca^{2+} in the thylakoid membrane following EGTA-treatment. Indeed, when the restoration by bovine serum albumin was carried out in the absence of EGTA, its amplitude was identical regardless of the presence or absence of Ca^{2+}. On the other hand, when EGTA was added to stop the phospholipase action, the amplitude of the restoration by bovine serum albumin was further enhanced by the addition of Ca^{2+}, which likely replenished the endogenous Ca^{2+} pool. The importance of Ca^{2+} in Photosystem II activity has been pointed out recently [28].

The results presented in Figs. 1–3 deal with non-cyclic electron flow activities. Similar results were obtained when Photosystem II activity (H_2O/DCIP in the presence of dibromothymoquinone) was measured, e.g., the inhibition in the presence of phospholipase A_2 and the partial restoration of the activity by bovine serum albumin (results not shown). Thus, for Photosystem II also, the bovine serum albumin-insensitive inhibition of the activity must be ascribed to the phospholipid depletion of the thylakoid membrane. In the case of Photosystem I ($DCIPH_2$/methyl viologen-O_2), the activities were stimulated (around 30%) by phospholipase A_2 (results not shown) in agreement with other reports [2,29], but the addition of bovine serum albumin suppressed this stimulation. This demonstrates that the phospholipase A_2-stimulation can be ascribed to the presence of hydrolysis products in the membrane as suggested by earlier studies [12,17]. However, even at a high degree of phospholipid depletion (e.g., after 80 min, see Table I) was Photosystem I activity, as measured by $DCIPH_2$/methyl viologen-O_2, not affected. Thus, in contrast to Photosystem II activity, Photosystem I is insensitive to the depletion of most phospholipid molecules. However, the very small amount of phospholipids remaining in the membrane (see Table I) may well be sufficient to support full Photosystem I electron-transport activity.

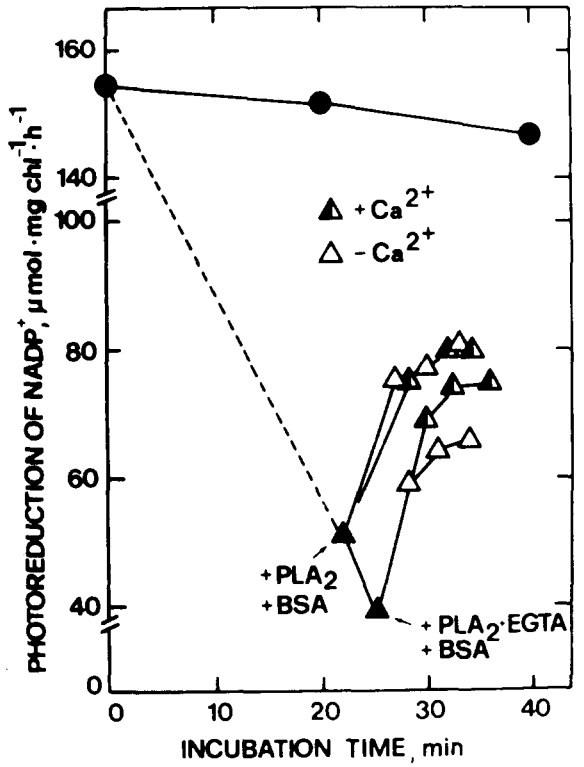

Fig. 3. Effect of Ca^{2+} on the photoreduction of $NADP^+$ in phospholipase A_2 (PLA_2)-treated samples which had been subsequently treated by bovine serum albumin (BSA) (20 mg/ml) alone or EGTA (4 mM) + bovine serum albumin. General experimental conditions and symbols are as in Figs. 1 and 2. After dilution, the reaction mixture for the determination of electron flow activity contained 0.8 mM EGTA and 2 mM $CaCl_2$.

In order to understand better the relationship between phospholipid depletion and the irreversible inhibition of electron-flow activity, we have plotted the extent of activity inhibition (in the presence of bovine serum albumin) as a function of lipid depletion (calculations are based on the results of Fig. 1 and Table I). Fig. 4 shows that for both phosphatidylcholine and phosphatidylglycerol, there are three lipid populations. These populations must be understood only as 'functional' lipid populations and not necessarily in terms of transmembrane distribution, although they are obviously part of the outer or inner (or both) monolayers. The first one (corresponding to about 40% of each phospholipid class) can be removed without affecting greatly the non-cyclic electron-flow activity (up to 10% inhibition). It is likely that most of these lipids belong to the outer monolayer, as suggested by previous results [2,4]. The second population of phospholipids (from 40

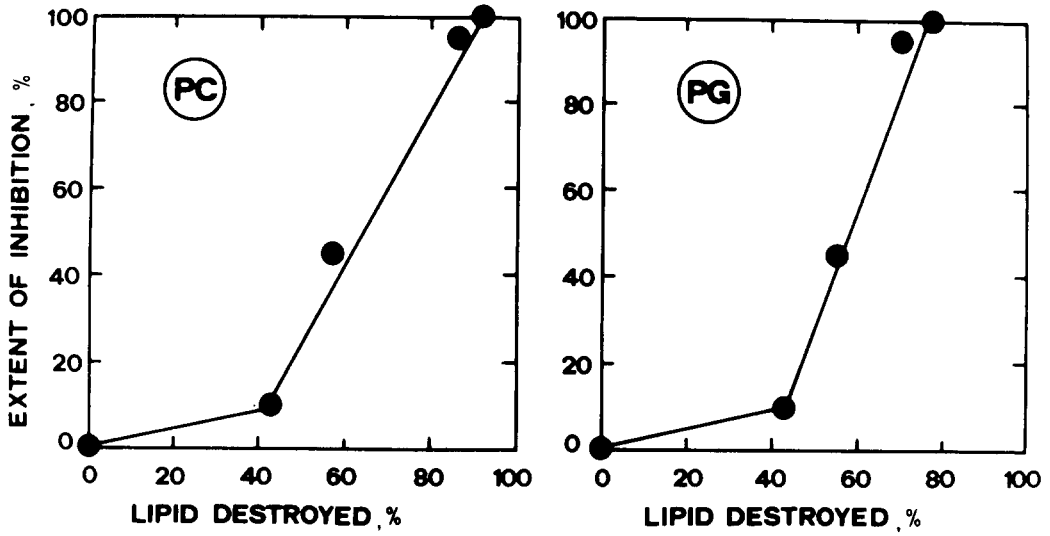

Fig. 4. Phospholipid dependency of non-cyclic electron flow activity. The extent of activity inhibition and phosholipid depletion are computed from the data of Table I and Fig. 1.

to 90% for phosphatidylcholine and from 40% to 80% for phosphatidylglycerol) is of utmost importance to sustaining the activity. The linear dependency of the activity towards this phospholipid population represents the searched relationship. It is noteworthy that this correlation appears only when those phospholipid molecules which are not required for sustaining the measured activity have been removed. It is therefore not surprising that previous studies using phospholipases failed to establish such a correlation, although they generally agreed that Photosystem II activity is inhibited, whereas Photosystem I is not impaired or slightly stimulated [30,31]. However, using phospholipase A_2 from snake venom of Habu, Hirayama and Nomotobori [32] have already suggested the existence of phospholipid population which can be removed from thylakoid membrane without significantly affecting several Photosystem II-mediated activities. Concerning this second lipid population (Fig. 4), it is likely that phosphatidylcholine molecules originate from both the outer and inner monolayers (via transbilayer movement), whereas phosphatidylglycerol molecules arise essentially from the outer monolayer [2,4,33]. Finally, the third phospholipid population (up to 10% for phosphatidylcholine and 20–30% for phosphatidylglycerol), which is not yet hydrolyzed by the phospholipase A_2, does not appear to support non-cyclic electron flow activity. This population belongs entirely to the inner monolayer of the thylakoid membrane. It is noteworthy that the phosphatidylglycerol content of this last population is entirely accounted for by the inner topological pool of this lipid [2,4,33].

The present results show that only one of the three above identified phospholipid populations is directly involved in sustaining uncoupled Photosystem II and non-cyclic electron-flow activities. Further experiments are in progress to assign a role to the other populations of phospholipids. In addition, it is interesting to mention that not only phospholipid depletion (this report), but also phospholipid enrichment [34,35] of the thylakoid membrane adversely affect electron flow activities. This suggests that the phospholipid content of the thylakoid membrane must be maintained between narrow limits to support optimal electron-flow activity.

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