Platelet Phospholipids Are Differentially Protected Against Oxidative Degradation by Plasmalogens

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ABSTRACT: The oxidative degradation of phospholipids in the presence and absence of plasmalogens (plasmenyl phosphatidylethanolamine: PPE) was followed by chemical analysis. Human platelet phospholipids, either intact or after removal of PPE by acid treatment, were oxidized with 28 mM 2,2′-azobis(2-amidinopropane di-HCl in Triton X-100 micelles (detergent/phospholipid 5:1, mol/mol). PPE (12% of all phospholipids, mol/mol) disappeared about three times more rapidly than glycerophospholipids, whereas sphingomyelin remained unaltered and the lysophosphatidylethanolamine (lysoPE) generated became progressively more unsaturated. After 60 min oxidation, the FA compositions of PS, PC, and PI were similar in extracts with or without plasmalogens. In contrast, diacyl phosphatidylethanolamine (DPE) became more saturated in the absence of PPE. The rate of phospholipid destruction was always unique to each class, but for all phospholipids slowed down in the presence of PPE. This protective effect increased in the order DPE < PS < PC < PI and did not seem to be simply related to the class unsaturation. α-Tocopherol had no influence on the time courses of the quantities and compositions of the phospholipids, even at a molar ratio of α-tocopherol to phospholipids four times higher than in platelet membranes. Thus, PPE protected phospholipids efficiently but differentially against peroxidative attack, whereas the contribution of α-tocopherol appeared to be negligible even at a concentration four times greater than in platelet membranes.

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Several findings suggest that oxygen free radicals behave as stimulators of platelet aggregation, acting at different levels of the signal activation pathways (1,2). There is also a growing body of evidence to support the role of these active radicals in a number of pathological situations involving platelet activation, such as atherosclerosis (3) or reperfusion injury (4). Since the early demonstration of a preferential attack of "active" oxygen on the vinyl-ether linkage of plasmalogen phospholipids (5), further reports have indicated that these membrane components can play an important role in antioxidant defense (3,6,7). The most convincing systems are those employing cultured cells (8,9), LDL particles (10–12), liposomes (13–15), or micelles (12,16). The plasmalogen content of platelets is relatively high, as these compounds constitute more than half the PE pool, thus accounting for about 12% of all membrane phospholipids. The contribution of choline plasmalogens nevertheless remains marginal and amounts to no more than 0.8% of the PC pool (17). Although many reports have described the susceptibility of plasmalogens to various oxidative reactions (5,9,13,14), there are no studies concerning their specific protection of phospholipids. The aim of the present work was to evaluate the protection of individual platelet phospholipids by plasmalogens during peroxidative attack by estimating their concentrations and compositions in an *in vitro* system.

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Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) di-HCl; DPE, diacyl phosphatidylethanolamine; lysoPE, lysophosphatidylethanolamine; PPE, plasmenyl phosphatidylethanolamine (plasmalogens); SM, sphingomyelin.

MATERIALS AND METHODS

Washed human platelets were prepared from the blood of four pools of healthy donors (18), and platelet lipids were extracted immediately (19). Tocopherols were stripped from the samples down to undetectable levels by filtering chloroform solutions onto silica gel; after eluting neutral lipids with chloroform, phospholipids were eluted with methanol. Aliquots of total phospholipids (about 9 μg P, final concentration 600 μM) were mixed with 3 mM Triton X-100 (12) in PBS (0.5 mL), and peroxidation was induced by addition of 2,2′-azobis(2-amidinopropane) di-HCl (AAPH) at a final concentration of 28 mM (Polysciences Inc., Eppelheim, Germany). Two micellar systems were used; in one, the phospholipids were left intact, whereas in the other plasmalogens were destroyed by vortexing chloroform solutions in the presence of 0.4 M HCl for 15 min (20). Free aldehydes were removed by filtration on a silica gel column, and phospholipids were eluted as described above. To compare the protective efficiency of plasmalogens and α-tocopherol, the oxidative degradation of phospholipids was also studied after addition of α-tocopherol (final concentration 2.8 or 11.2 μM; Sigma Chemical Co., Lyon, France) before AAPH to a phospholipid suspension without plasmalogens. In these experiments, the molar ratios of phospholipids to α-tocopherol were similar to those in platelet membranes (about 214:1) for 2.8 μM or up to fourfold lower (about 54:1) for 11.2 μM α-tocopherol. After incubation at 37°C for 0, 15, 30, 45, or 60 min, phospholipids were extracted with a Folch mixture. PE, PC, PS, PI, and sphingomyelin (SM) were separated by TLC (21), and their FA compositions were analyzed by GLC after direct transmethylation with BF_3/methanol reagent (21). Addition of a known amount of heptadecanoic acid as an internal standard enabled estimation of the mass of each phospholipid. DPE and PPE were quantified after elution of the PE spots

and cleavage of the vinyl bond by acid treatment (21), the lysoPE representative of PPE and the remaining DPE being separated by a second TLC run. α-Tocopherol was estimated by HPLC with fluorescence detection (19).

The Mann–Whitney test was used to compare the means between experimental groups.

RESULTS

In the present study, the phospholipid composition of the platelet samples was similar to that previously reported (21) using the same analytical procedure. The average composition of the phospholipid mixture was (mol %): PPE (14.2 ± 1.1), DPE (12.3 ± 0.6), PC (46.3 ± 2.6), PS (12.3 ± 0.5), PI (4.7 ± 0.2), and SM (10.2 ± 0.6).

Exposure of intact platelet phospholipids to 28 mM AAPH led to a progressive loss of diacylphospholipids, which were at 25% of their initial level after 60 min oxidation. The AAPH concentration was selected to obtain a degradation rate similar to that previously observed in brain phospholipid mixtures (16). The destruction of PPE was more rapid than that of other phospholipids, occurring mainly within the first 15 min and reaching a loss of about 75% after 60 min. Moreover, the FA composition of the remaining PPE was significantly altered (Table 1). The new FA profile after 60 min oxidation was characterized by important increases in saturated and n-9 FA, compensated by important decreases in n-6 and n-3 PUFA of, respectively, 24 and 45%. The lysoPE generated amounted to about 7% of total diacylphospholipids, and its FA composition differed significantly from that of the small amount present at time 0 (less than 1%). The concentration of arachidonic acid was four times its value at time 0, while an increase of n-6 and n-3 PUFA compensated for a decrease of n-9 FA. The FA compositions of DPE, PS, PC, and PI at time 0 and after 60 min of oxidation in the presence or absence of plasmalogens are given in Tables 2 and 3. AAPH induced important losses of PUFA (14% for DPE and about 27% for PS, PC, and PI) in intact phospholipid extracts (with plasmalogens), compensated by increases in the concentrations of saturated and monounsaturated FA. The FA compositions of PS (Table 2), PC, and PI (Table 3) in intact extracts were similar to those in extracts without plasmalogens after 60 min oxidation. In contrast, the composition of DPE was less affected by AAPH in the presence of plasmalogens (Table 2). Thus, plasmalogens significantly reduced the losses of n-6 PUFA (13 vs. 32%, $P < 0.01$) and MUFA (13 vs. 35%, $P < 0.001$), whereas saturated FA

TABLE 1
Effects of AAPH on the FA Composition of Plasmenylethanolamine and Lysophosphatidylethanolamine in Intact Platelet Extracts[a]

	PPE		LysoPE	
	0	60 min	0	60 min
14:0	0.2 ± 0.0	2.2 ± 0.1***	6.0 ± 0.3	0.8 ± 0.0***
16:0	1.6 ± 0.1	10.0 ± 0.4***	22.3 ± 1.3	8.7 ± 0.5***
18:0	1.4 ± 0.1	9.9 ± 0.4***	24.5 ± 1.2	28.8 ± 1.5
Sum Sat	3.2 ± 0.2	22.0 ± 1.2***	52.8 ± 3.3	39.0 ± 2.1*
16:1n-9	0.5 ± 0.0	3.5 ± 0.2***	7.0 ± 0.3	2.8 ± 0.2***
18:1n-9	1.7 ± 0.1	5.1 ± 0.3***	11.1 ± 0.6	6.7 ± 0.4***
20:2n-9	0.7 ± 0.0	0.0 ± 0.0	6.5 ± 0.3	1.3 ± 0.0***
Sum n-9	2.9 ± 0.2	8.6 ± 0.4***	24.6 ± 1.8	11.0 ± 0.5***
18:1n-7	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
Sum n-7	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.7 ± 0.0
18:2n-6	1.1 ± 0.0	1.7 ± 0.0	1.0 ± 0.0	1.9 ± 0.1
18:3n-6	0.6 ± 0.0	2.0 ± 0.1***	9.6 ± 0.5	1.5 ± 0.0
20:2n-6	0.1 ± 0.0	1.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:3n-6	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.0
20:4n-6	63.2 ± 3.4	44.9 ± 2.0**	9.2 ± 0.4	34.0 ± 1.9***
22:4n-6	16.8 ± 0.8	12.3 ± 0.7**	1.4 ± 0.0	8.8 ± 0.6***
22:5n-6	0.9 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	0.4 ± 0.0
Sum n-6	83.3 ± 4.3	63.4 ± 3.9**	21.8 ± 1.5	47.1 ± 2.6 ***
20:5n-3	0.5 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.2 ± 0.0
22:5n-3	5.5 ± 0.3	3.3 ± 0.2**	0.5 ± 0.0	1.2 ± 0.0
22:6n-3	4.6 ± 0.3	2.3 ± 0.1***	0.0 ± 0.0	0.7 ± 0.0
Sum n-3	10.6 ± 0.5	5.8 ± 0.4***	0.5 ± 0.0	2.1 ± 0.1**
MUFA	2.3 ± 0.1	8.7 ± 0.6***	18.4 ± 1.2	10.5 ± 0.7***
PUFA	94.5 ± 5.9	69.2 ± 4.6*	28.7 ± 1.9	50.5 ± 3.5**

[a]Results are mean ± SEM ($n = 4$). AAPH, 2,2′-azobis(2-amidinopropane) di-HCl; lysoPE, lysophosphatidylethanolamine; MUFA, monounsaturated FA; PPE, plasmenylethanolamine (plasmalogens); Sum Sat, sum of the saturated FA; Sum n-9, sum of n-9 FA; Sum n-7, sum of n-7 FA; Sum n-6, sum of n-6 FA; Sum n-3, sum of n-3 FA. PPE amounted to 12.5% (mol/mol) of the total phospholipids at time 0 and 3.9% after 60 min; lysoPE amounted to 1% at time 0 and 6.9% after 60 min. The Mann–Whitney test was used to compare the means at 0 and 60 min for each compound: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

TABLE 2
Effects of AAPH on the FA Composition of Diacyl PE and PS in Platelet Extracts With and Without PPE[a]

	DPE			PS		
	0	C	AT	0	C	AT
16:0	8.5 ± 0.5	10.6 ± 0.5	6.6 ± 0.3***	2.6 ± 0.1	4.6 ± 0.2	3.0 ± 0.1***
18:0	34.7 ± 1.8	35.7 ± 1.6	54.5 ± 2.9**	44.2 ± 2.1	46.4 ± 2.2	52.7 ± 2.7
20:0	1.3 ± 0.1	1.4 ± 0.1	0.0 ± 0.0	1.6 ± 0.0	1.8 ± 0.0	2.1 ± 0.1
Sum Sat	44.9 ± 2.3	48.5 ± 2.5	61.5 ± 3.1*	49.4 ± 2.3	54.2 ± 3.0	58.0 ± 3.2
16:1n-9	0.4 ± 0.0	0.9 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	1.4 ± 0.0	0.5 ± 0.0
18:1n-9	6.4 ± 0.3	8.8 ± 0.4	5.4 ± 0.3***	16.1 ± 0.8	19.0 ± 0.8	16.6 ± 0.6
20:1n-9	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:2n-9	0.3 ± 0.0	0.6 ± 0.0	0.0 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
Sum n-9	7.6 ± 0.3	10.8 ± 0.4	6.1 ± 0.3***	17.5 ± 0.9	21.4 ± 1.0	17.9 ± 0.8
16:1n-7	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
18:1n-7	1.0 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.6 ± 0.0
Sum n-7	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.6 ± 0.0
18:2n-6	4.2 ± 0.2	5.2 ± 0.3	3.0 ± 0.1***	0.6 ± 0.0	0.9 ± 0.0	0.7 ± 0.0
18:3n-6	0.5 ± 0.0	1.0 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.7 ± 0.0
20:2n-6	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
20:3n-6	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	1.2 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
20:4n-6	34.6 ± 1.6	27.9 ± 1.5	22.5 ± 1.0*	26.2 ± 1.2	17.7 ± 0.9	18.4 ± 0.8
22:4n-6	3.2 ± 0.1	3.0 ± 0.1	2.9 ± 0.2	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0
22:5n-6	0.4 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Sum n-6	43.8 ± 2.0	38.0 ± 1.8	29.7 ± 1.6**	30.9 ± 1.5	22.2 ± 1.1	22.6 ± 1.0
22:5n-3	1.1 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
22:6n-3	1.5 ± 0.0	0.8 ± 0.0	0.7 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	0.4 ± 0.0
Sum n-3	2.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.0	1.2 ± 0.0	0.9 ± 0.0
MUFA	8.3 ± 0.4	11.2 ± 0.5	7.2 ± 0.3***	17.6 ± 0.8	21.7 ± 1.0	18.0 ± 0.7*
PUFA	46.8 ± 2.6	40.2 ± 2.1	31.3 ± 1.4*	33.0 ± 1.7	24.1 ± 1.1	23.9 ± 1.0
DBI	189.5 ± 10.0	161.7 ± 8.3	127.0 ± 6.9*	147.0 ± 8.1	115.1 ± 5.4	111.2 ± 5.0

[a]Results are mean ± SEM ($n = 4$). DPE, diacyl PE; 0, extracts at time 0 before addition of AAPH; C, control extracts with plasmalogens after 60 min oxidation; AT, acid-treated extracts (without plasmalogens) after 60 min oxidation; DBI, double bond index (number of double bonds for 100 mol FA). Other abbreviations as in legend of Table 1. The Mann–Whitney test was used to compare the means of C and AT extracts: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

were correspondingly more abundant in extracts without plasmalogens (37%) than in intact extracts (8%) ($P < 0.05$).

The time courses of the relative quantities of the phospholipids and their PUFA contents during AAPH oxidation are shown in Figure 1. In intact extracts, the rate of phospholipid destruction was constant over 1 h but differed according to the class, with only 40% of the initial DPE remaining after 60 min as compared to about 75% of PS and PI and 87% of PC. In the absence of plasmalogens, all phospholipids disappeared rapidly over the first half hour but more slowly thereafter, the destruction of DPE and PI being strongest (26–29% remaining) and that of PS and PC weakest (48–50% remaining). The time courses of the relative PUFA contents of all phospholipids were similar to those of their quantities in extracts with or without plasmalogens. In the absence of plasmalogens, the addition of α-tocopherol (2.8 or 11.2 μM) before AAPH did not alter the time courses of the quantities and compositions of the phospholipids in the incubation mixture (data not shown). At both concentrations, α-tocopherol was no longer detectable after 15 min oxidation in the presence of AAPH.

DISCUSSION

Although the oxidative degradation of plasmalogens has been accurately described (11,14,22), their role in the protection of diacyl phospholipids remains poorly understood. The effects of plasmalogens on the oxidative decay of total tissue phospholipids (15) or of certain specific molecular species (12,16) have been reported, but comparative effects on the degradation of individual phospholipids have not yet been described. As previously observed in various systems (14–16), there was a pronounced loss of PPE following exposure of intact platelet phospholipids to AAPH, as compared to the loss of total diacyl phospholipids. Furthermore, we observed an accumulation of lysoPE-bearing PUFA groups (50.5 ± 5.2% after 60 min). This specific destruction of PPE species having a polyunsaturated fatty acyl group esterified at the *sn*-2 position is in accordance with earlier reports (22). In contrast, an accumulation of lysoPE molecular species containing only monounsaturated fatty acyl groups was observed after oxidation of bovine brain PE (14). This discrepancy could be related to different experimental conditions and/or to the use of only PE (14) instead of a phospholipid mixture (present work). On a mass basis, the results show that plasmalogens strongly protect all diacyl phospholipids (Fig. 1). In the presence of plasmalogens (nontreated extracts), all phospholipids and their PUFA disappeared at a constant rate over a period of 1 h, whereas without plasmalogens (acid-treated extracts) the degradation proceeded more rapidly. Similar kinetics have been described by others in liposomal (15) and micellar

TABLE 3
Effects of AAPH on the FA Composition of PC and PI in Platelet Extracts With and Without PPE[a]

	PC			PI		
	0	C	AT	0	C	AT
16:0	31.9 ± 1.5	33.4 ± 1.4	32.7 ± 1.3	3.0 ± 0.2	9.4 ± 0.5	5.6 ± 0.3**
18:0	15.4 ± 0.7	18.4 ± 0.8	18.1 ± 0.9	43.1 ± 2.0	44.5 ± 2.1	48.8 ± 2.4
20:0	1.2 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	1.0 ± 0.0
Sum Sat	49.0 ± 2.5	53.6 ± 2.8	52.4 ± 3.2	47.0 ± 2.1	55.9 ± 2.6	56.0 ± 2.6
16:1n-9	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	2.5 ± 0.1	1.0 ± 0.0
18:1n-9	17.8 ± 0.8	20.4 ± 1.1	20.6 ± 1.2	2.0 ± 0.1	4.4 ± 0.2	4.1 ± 0.2
20:1n-9	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:2n-9	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.9 ± 0.0
Sum n-9	18.9 ± 0.9	21.7 ± 1.0	22.0 ± 1.1	3.3 ± 0.2	7.8 ± 0.5	6.2 ± 0.4
16:1n-7	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
18:1n-7	2.0 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
Sum n-7	2.6 ± 0.1	3.0 ± 0.1	2.8 ± 0.1	0.5 ± 0.0	0.8 ± 0.0	0.9 ± 0.0
18:2n-6	9.1 ± 0.4	8.6 ± 0.4	8.7 ± 0.4	0.5 ± 0.0	1.1 ± 0.0	1.2 ± 0.0
18:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0
20:2n-6	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:3n-6	1.4 ± 0.1	1.1 ± 0.0	1.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
20:4n-6	15.8 ± 0.6	9.6 ± 0.5	10.4 ± 0.5	46.5 ± 2.3	31.6 ± 1.7	32.6 ± 1.9
22:4n-6	1.2 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.4 ± 0.0	1.0 ± 0.0	1.1 ± 0.0
22:5n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sum n-6	28.3 ± 1.4	21.1 ± 1.0	22.1 ± 1.3	49.0 ± 2.4	35.4 ± 1.9	36.5 ± 2.6
22:5n-3	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
22:6n-3	0.6 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
Sum n-3	1.2 ± 0.1	0.6 ± 0.0	0.8 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
MUFA	21.4 ± 1.0	24.6 ± 1.2	24.6 ± 1.3	3.0 ± 0.1	7.9 ± 0.4	6.2 ± 0.4
PUFA	29.6 ± 1.2	21.8 ± 0.9	23.0 ± 1.1	50.0 ± 2.8	36.2 ± 2.2	37.8 ± 2.4
DBI	120.8 ± 6.2	92.7 ± 4.8	97.4 ± 5.3	199.2 ± 8.6	147.9 ± 9.2	152.2 ± 10.1

[a]Results are mean ± SEM (n = 4). For abbreviations see Tables 1 and 2. The Mann–Whitney test was used to compare the means of C and AT extracts: **$P < 0.01$.

systems (16). Comparing the degradation rates of the different diacyl phospholipids and their PUFA over the first 30 min, we found that the protective effect of plasmalogens increased in the order DPE < PS < PC < PI. Thus, the degradation after 30 min oxidation decreased in the presence of plasmalogens by 48 ± 3, 66 ± 4, 85 ± 4, and 88 ± 5% for DPE, PS, PC, and PI, respectively. Among DPE, PS, and PC, the efficiency of protection was inversely related to the double bond index (respectively, 189 ± 10, 147 ± 8, and 121 ± 6) or PUFA content (respectively, 46.8 ± 2.6, 33.0 ± 1.7, and 29.6 ± 1.2%), the most unsaturated phospholipid (DPE) being least efficiently protected by plasmalogens. Unexpectedly, although PI is still more unsaturated than DPE, it appeared to be the most strongly protected of the diacyl phospholipids (Fig. 1).

These results demonstrate that plasmalogens participate actively in the inhibition of PUFA peroxidation and concomitantly in phospholipid degradation, the protection afforded depending on both the acyl chain unsaturation and the nature of the polar head. Interestingly, in the absence of plasmalogens the degradation rates of the four glycerophospholipids increased in the order PS < PC < DPE < PI, which is not the order of their increasing unsaturation (PC < PS < DPE < PI). Hence, if unsaturation is important for peroxidative susceptibility (23), there is also an obvious influence of the polar head group among the diacyl glycerophospholipids. Moreover, the least unsaturated phospholipid (SM) was unaffected by free radical oxidation in our experimental system, even in the absence of plasmalogens (data not shown).

In natural membranes, α-tocopherol is currently considered to be the major lipid-soluble antioxidant despite its low concentration, which approximates 1 mole for 220 moles of phospholipids, as for instance in platelets (19). The relative potency of α-tocopherol as compared to plasmalogens as a protective agent against phospholipid degradation induced by peroxyl radicals was evaluated by adding α-tocopherol before AAPH to membrane extracts without plasmalogens. We observed that the rates of phospholipid degradation remained unchanged when using molar ratios of phospholipids to α-tocopherol similar to that found in platelet membranes (214:1) or up to about fourfold lower (54:1). These unexpected results are probably at least partly due to total consumption of the added α-tocopherol after 15 min oxidation. Although a significant decrease in degradation rate was previously reported (16), these authors used a higher α-tocopherol amount (4:1 ratio of phospholipid to α-tocopherol) and a lower AAPH concentration (2 mM) than in our experiments. Thus, in our conditions, plasmalogens appear to contribute more effectively than α-tocopherol to the resistance of the unsaturated acyl chains of phospholipids to peroxidation. In spite of a scavenging activity estimated to be similar to (10) or fourfold higher (12) than that of plasmalogens, in platelet membranes only 1 mole of α-tocopherol is able to compete with about 30 moles of plasmalogens

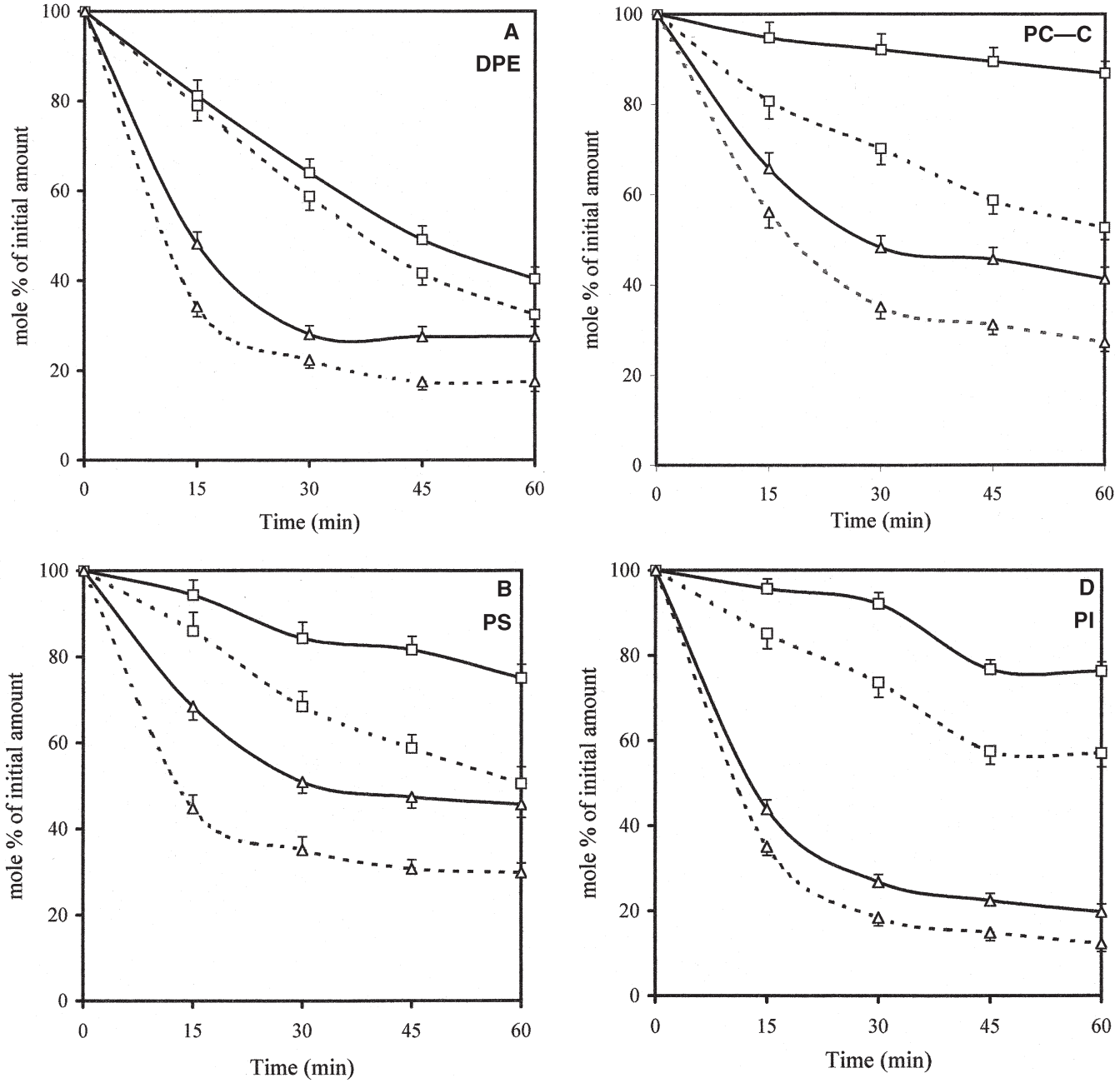

FIG. 1. 2,2′-Azobis(2-amidinopropane) di-HCl–mediated degradation of platelet phospholipids in the presence or absence of plasmalogens. Mean values (± SEM) in four independent experiments where the mass values obtained at time 0 were set to 100%. (A) diacyl phosphatidylethanolamine, (DPE) (B), PS; (C), PC; (D), PI. Relative mass of a given phospholipid in control extracts (with plasmalogens) (–□–), and in acid-treated extracts (without plasmalogens) (–△–); relative PUFA content of a given phospholipid in control extracts: (--□--), and in acid-treated extracts (without plasmalogens) (--△--).

for interaction with peroxyl radicals. Any extrapolation of the present findings to biological membranes will nevertheless require further work to clarify the mechanisms responsible for the differential protection of phospholipids by plasmalogens.

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