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Acyl Chain and Head Group Regulation of Phospholipid Catabolism in Senescing Carnation Flowers[1]

Jacqueline H. Brown, James A. Chambers, and John E. Thompson*

Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

ABSTRACT

Microsomal membranes from the petals of senescing carnation (*Dianthus caryophyllus* L.) flowers contain phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol. These phospholipid classes decline essentially in parallel during natural senescence of the flower and when microsomal membranes isolated from young flowers are aged *in vitro*. However, measurements of changes in the endogenous molecular species composition of microsomal phospholipids during natural senescence of the flower petals and during *in vitro* aging of isolated membranes have indicated that the various molecular species of phospholipids have quite different susceptibilities to catabolism. Acyl chain composition and the nature of the head group are both determinants of their susceptibility to catabolism. As well, a comparison of the phospholipid catabolism data for naturally senesced membranes and for membranes aged *in vitro* suggests that the phospholipid composition of membranes is continuously altered during senescence by acyl chain desaturation and possibly retailoring so as to generate molecular species that are more prone to catabolism. The results collectively indicate that provision of particular molecular species of phospholipids with increased susceptibility to degradation contributes to enhanced phospholipid catabolism in the senescing carnation petal.

Loss of membrane integrity is an early and fundamental feature of senescence in plant tissues, and perhaps the clearest manifestation of this is the onset of leakiness attributable to increased membrane permeability. In petals of cut carnation flowers, for example, solute leakage is detectable before petal inrolling and the climacteric-like rise in ethylene production, symptoms that are thought to denote the initiation of petal senescence (7). Studies with various techniques including electron spin resonance (20), fluorescence depolarization (9), freeze-fracture electron microscopy (24), and x-ray diffraction (19) have indicated that there is also a decrease in membrane lipid fluidity with advancing senescence. This appears to reflect the formation of domains of gel phase lipid as well as a decrease in bulk lipid fluidity attributable to an increased relative concentration of free sterols in the bilayer (29).

There is also extensive catabolism of membrane phospholipids during senescence. This has been demonstrated for senescing flower petals (9, 27), senescing leaves and cotyledons (8, 20), and ripening fruit (18) and results in a substantial increase in the sterol:phospholipid ratio of membranes as senescence progress (9, 29). Several phospholipid degrading enzymes have been identified in plant tissues including phospholipase D (13), lipolytic acyl hydrolase (10), and phospholipase C (14), and recent evidence suggests that phospholipase D may initiate phospholipid catabolism in senescing membranes (5). Although there is clear evidence for a net decline in phospholipid levels with advancing senescence, the extent to which this reflects decreased synthesis, enhanced catabolism, or possibly both has not been resolved. An age-related reduction in phospholipid synthesizing ability has been demonstrated in a number of plant tissues (3). Increased lipid catabolism during senescence has also been observed, but under conditions in which there is no absolute increase in phospholipase activity (27). This suggests that in at least some senescing tissues enhanced degradation of phospholipid may be attributable to alterations in membrane lipids that make them more susceptible to attack by lipid-degrading enzymes. Perturbed phospholipid bilayers, such as those containing a mixture of lipid phases, are known to be degraded at a higher rate by phospholipase A_2 than bilayers containing exclusively liquid crystalline phase lipid (11), and platelet hydrolysis by endogenous phospholipase A_2 has been shown to be facilitated by decreased membrane fluidity as well as elevated cholesterol levels (15). It is noteworthy in this context that decreased bilayer fluidity, a mixture of lipid phases and enhanced cholesterol levels are all characteristic features of senescing membranes (29).

In this present study, we have examined factors regulating phospholipid catabolism in the petals of senescing carnation flowers. The data indicate that the head group and fatty acyl chain composition of phospholipids are both determinants of their susceptibility to catabolism during senescence and that provision of phospholipid molecular species with enhanced susceptibility to catabolism may be a regulatory feature of phospholipid catabolism in senescing membranes.

MATERIALS AND METHODS

Plant Material

Carnation flowers (*Dianthus caryophyllus* L. cv White Sim) were grown in a commercial greenhouse (William Hofland Greenhouses Ltd., Beamsville, Ontario). They were cut at a young stage when the petals had expanded approximately 2 cm beyond the sepals. The stems were trimmed to a length of 22 cm, and the flowers were placed individually in glass culture tubes containing deionized water. They were main-

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tained at 22°C under continuous illumination (irradiance 10 W m^{-2}; Sylvania, Seneca Falls, NY) until they had reached specific stages of senescence, *viz.* stage II, flowers that still possessed yellowish-tinted centers, but were fully expanded; and stage IV, senescent flowers showing petal-inrolling.

Membrane Isolation

Microsomal membranes were isolated from the petals of stage II and stage IV flowers in 20 mM EPPS[2] (pH 7.3) as described previously (30) and washed once by resuspension in the same buffer and centrifugation at 131,000*g* for 1 h. The resulting pellet was resuspended in 3 mL of 70 mM EPPS (pH 7.3) and dialyzed at 4°C against three changes of 600 mL of 2 mM EPPS (pH 7.3) for a total of 15 h. After dialysis, the protein concentration was adjusted to 1 mg mL^{-1} with 70 mM EPPS (pH 7.3). Protein was measured as described by Bradford (4) using bovine serum albumin as a standard.

Molecular Species Analysis

Changes in the molecular species composition of endogenous phospholipids in microsomal membranes were measured during natural senescence and also during *in vitro* aging of isolated membranes. For the *in vitro* reaction, three 5 mL aliquots of washed, dialyzed membrane suspension (1 mg protein mL^{-1}) were placed in separate 40 mL Pyrex tubes. One sample served as the zero-time control, and the other two were incubated for 1.5 and 3 h, respectively, at 30°C in darkness. The reactions were terminated by lipid extraction (1). For measurements of changes in molecular species composition during natural senescence, 5 mL samples of nondialyzed membrane suspension (1 mg protein mL^{-1}) obtained from stage II or stage IV flowers were lipid-extracted directly (1).

For molecular species analysis, the phospholipid classes in the total lipid extracts were separated by TLC on silica gel 60 plates using a development solvent of chloroform:methanol:acetic acid:water (80:15:15:3.5, v/v). The separated lipids were scraped from the plates and eluted from the silica gel in methanol:chloroform:water (2:1:0.8, v/v). Each purified class of phospholipids was converted to the corresponding diacylglycerols using phospholipase C (Boehringer Mannheim), derivatized with MSTFA (Pierce Chemical Co.) to form TMS derivatives and analyzed by GLC as described previously (17). The chromatography was performed using a Hewlett-Packard 5890 Gas Chromatograph equipped with a 15 m × 0.25 mm i.d. fused silica capillary column coated with dimethylpolysiloxane containing 50% cyanopropyl substitution (DB-23) (J & W Scientific). The column, injector, and detector temperatures were 248, 280, and 300°C, respectively. A split ratio of 20:1 was used, and the helium head pressure was maintained at 150 kPa.

The separated molecular species were identified by cochromatography with known molecular species (16:0/16:0, 16:0/18:1, 18:1/18:1, 18:0/18:0) derived from single purified phospholipids (Sigma) as well as by comparison with the known separation pattern of the molecular species of purified soybean PC (22). Confirmation of identity was obtained by comparing the expected fatty acid composition of the composite of separated molecular species, as determined from their assigned identities, with the measured fatty acid composition of the sn-1 and sn-2 positions of the purified phospholipids determined as described by Ramesha and Thompson (25). Fatty acid methylation was carried out according to Morrison and Smith (21). The methyl esters were separated isothermally with a split ratio of 120:1 on a 15 m × 0.25 mm i.d. fused silica capillary column coated with SP 2330 (Supelco). The column, injector, and detector temperatures were 175, 230, and 275°C, respectively. The separated methyl esters were identified by cochromatography with rapeseed oil standards.

Phospholipid phosphate was measured as described by Rouser *et al.* (26).

RESULTS

Microsomal membranes isolated from the petals of carnation flowers contain four classes of phospholipid, *viz.* PC, PE, PG, and PI. Phosphatidic acid is also present in these membranes but is thought to be only a transitory product of phospholipase D activity (23). A comparison of the phospholipid levels in microsomes isolated from young stage II flowers and senescent stage IV flowers indicated that the older membranes contain less total phospholipid relative to protein than the younger membranes. Microsomes from stage II flowers contained 247.7 ± 9.1 nmol phospholipid mg $protein^{-1}$ (SE for $n = 7$), whereas those from stage IV flowers contained 178.5 ± 2.7 nmol phospholipid mg $protein^{-1}$ (SE for $n = 7$). There was also a decline during senescence in each of the four classes of phospholipid found in the membranes on a per mg protein basis (Fig. 1A). However, the relative proportions of the phospholipid classes in the microsomal membranes did not change significantly as the flowers senesced (Fig. 1B) indicating that each class of phospholipid declined by essentially the same proportion.

Microsomal membranes from senescing carnation petals are able to catabolize exogenous radiolabeled phospholipids (5, 23), and thus the effects of *in vitro* incubation of microsomes isolated from young stage II flowers on phospholipid levels were also determined. During dialysis of the isolated membranes and the subsequent 1.5 and 3 h incubation periods, there were no significant changes in the relative proportions of PC, PE, PG, or PI (Fig. 2A). This, however, does not mean that the phospholipids were not catabolized. Indeed, the total phospholipid content of the microsomes decreased from 247.7 nmol/mg original protein before dialysis to 86.3 nmol/mg original protein after the 3 h incubation, but the different classes of phospholipid all declined essentially in parallel (Fig. 2B). PC and PE declined by 67.5 and 67.6%, respectively, whereas PG declined by 60.9% and PI by 72% (Fig. 2B). (These values were not significantly different at $P = 0.05$.) There was no decline for any of the phospholipid classes if the membranes were heat denatured before dialysis

[2] Abbreviations: EPPS, *N*-2-hydroxyethyl-piperazine-*N*'-3-propane sulphuric acid; MSTFA, *N*-methyl-*N*-TMS-trifluoroacetamide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; TMS, trimethylsilyl.

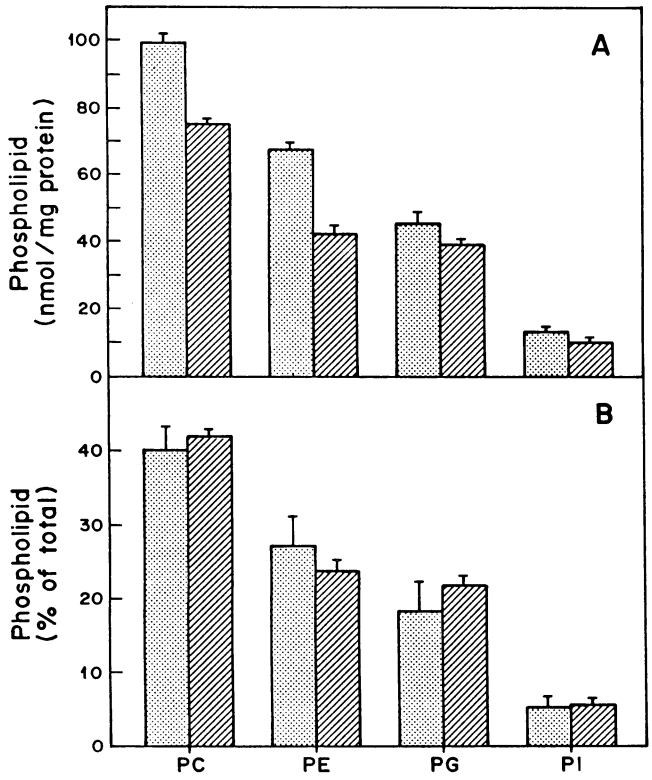

Figure 1. Phospholipid composition of microsomal membranes isolated from the petals of carnation flowers. Stippled bars, young stage II flowers; hatched bars, senescent stage IV flowers. A, Values are expressed as nmol phospholipid mg protein^{-1}; B, values are expressed as percentage of total phospholipid. Means ± SE are shown for n = 7–13.

and incubation, indicating that the degradation is enzymatically mediated.

These results collectively suggest that the classes of phospholipid in these membranes are not differentially sensitive to enzymatic degradation during natural senescence of the petals or when the catabolism occurs *in vitro* during incubation of isolated membranes. However, each phospholipid class is composed of different kinds and amounts of molecular species, and thus to more fully evaluate the effect of head group on susceptibility to degradation, levels of individual molecular species within each phospholipid class were measured during natural senescence and during *in vitro* incubation of isolated membranes.

During natural senescence, the PC content of the microsomal membranes declined from 99.2 nmol/mg protein for stage II flowers to 75 nmol/mg protein for stage IV flowers (Fig. 1A), although the relative amount of PC as a proportion of total phospholipid did not change (Fig. 1B). There were, however, changes in the molecular species composition of PC during senescence. Specifically, levels of 16:0/18:2 PC, 18:1/18:2 PE, 18:2/18:2 PC, and 16:0/18:1 PC declined on a per mg protein basis, and levels of the remaining molecular species relative to membrane protein remained essentially unchanged as the flowers senesced (Fig. 3A). The molecular species 18:0/18:0 PC was not detectable in membranes from stage II flowers but was clearly and reproducibly detectable by stage IV (Fig. 3A). Analysis of the changes in molecular species of PC during *in vitro* incubation revealed a different profile from that observed during natural senescence. The dominant molecular species, 16:0/18:2 and 18:2/18:2, declined in both instances, but the levels of 16:0/18:3 PC, 18:1/18:3 PC, and 18:2/18:3 PC declined during *in vitro* aging but remained unchanged during natural senescence (Fig. 3, A and B). As well, the molecular species 18:0/18:0 PC, which was detectable in membranes from stage IV flowers (Fig. 3A), was not formed during *in vitro* incubation of the membranes (Fig. 3B).

PE also remained an essentially constant proportion of the total microsomal phospholipid with advancing senescence even though its absolute level decreased from 67.4 to 42.2 nmol/mg membrane protein (Fig. 1, A and B). As well, the molecular species composition for PE resembled that for PC in that 16:0/18:2 and 18:2/18:2 were again dominant (Figs. 3 and 4). The two major molecular species of PE declined dramatically during natural senescence, and a number of molecular species, 16:0/16:0, 16:0/18:0, 16:0/18:3, 18:0/18:0, and 18:2/18:3, were formed during senescence inasmuch as they were not detectable in young stage II flowers and were clearly and reproducibly detectable in senescent stage IV

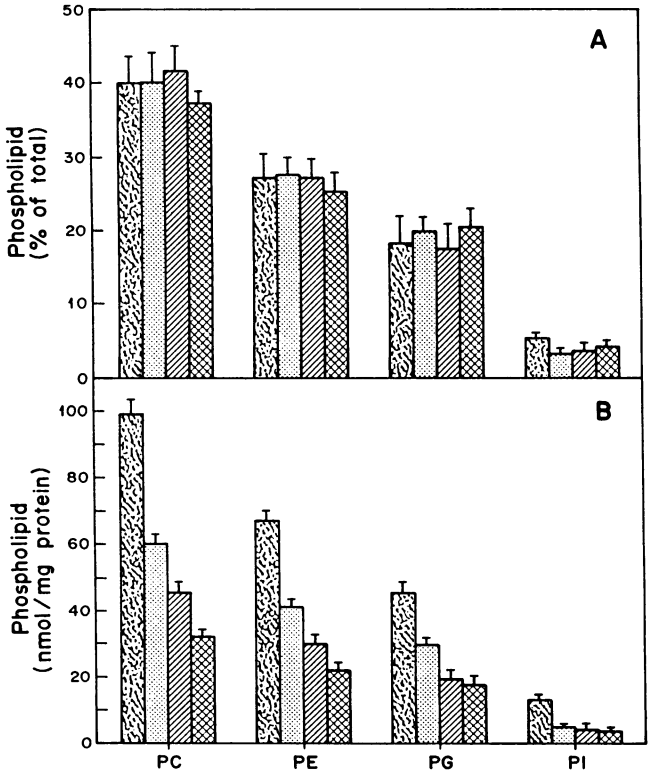

Figure 2. Changes in phospholipid composition during *in vitro* incubation of microsomal membranes isolated from the petals of young stage II carnation flowers. Bars left to right: nondialyzed control; dialyzed control at 0 h incubation; at 1.5 h incubation; at 3 h incubation. A, Values are expressed as percentage of total phospholipid; B, values are expressed as nmol phospholipid mg protein^{-1}. Means ± SE are shown for n = 7.

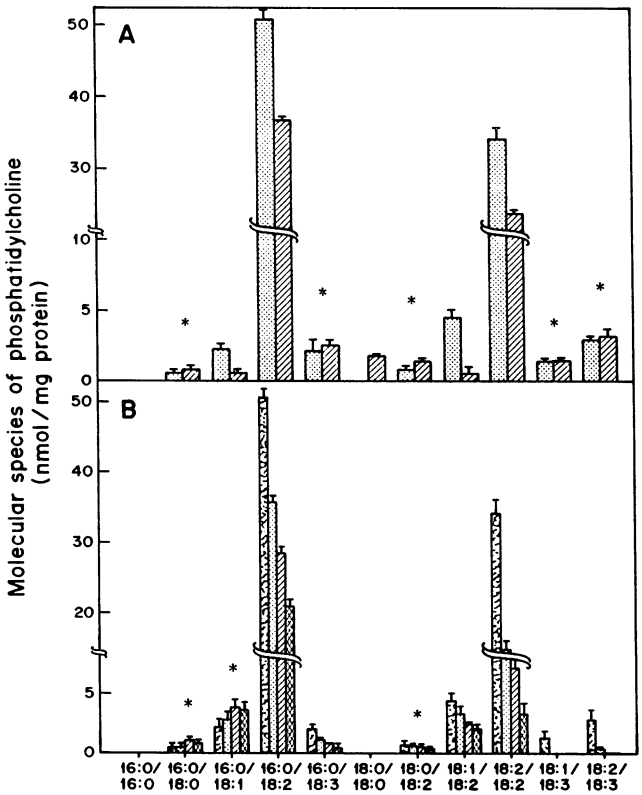

Figure 3. Molecular species composition of PC in microsomal membranes isolated from the petals of carnation flowers. A, Changes during natural senescence: left bars, young stage II flowers; right bars, senescent stage IV flowers. B, Changes during *in vitro* incubation of microsomal membranes from young stage II flowers. Bars left to right: nondialyzed control; dialyzed control at 0 h incubation; at 1.5 h incubation; at 3 h incubation. Means ± SE are shown for $n = 3$. Asterisks denote values that are not significantly different ($P = 0.05$).

flowers (Fig. 4A). During *in vitro* incubation, the two major molecular species again declined, but those molecular species showing enhanced levels during senescence (in the sense that they were detectable at stage IV but not at stage II) were not formed during *in vitro* incubation (Fig. 4B).

The molecular species composition of microsomal PG differed from those of PC and PE in that 16:0/16:0 and 16:0/18:2 rather than 16:0/18:2 and 18:2/18:2 were the predominant molecular species (Figs. 3, 4, and 5). The absolute levels of PG in the microsomal membrances declined during senescence from 45.3 to 39.2 nmol/mg protein for stage II and stage IV flowers, respectively (Fig. 1A). This decline can be accounted for mainly in terms of a pronounced drop in the major molecular species, 16:0/18:2 PG, from 29.2 nmol/mg protein to 15.1 nmol/mg protein (Fig. 5A). Distinctive features of the change during senescence in PG molecular species composition in comparison with corresponding changes for PC and PE were: (a) the large increase in 16:0/16:0 PG, which was already the second most abundant molecular species (Fig. 5A) (this molecular species is undetectable for PC and PE in membranes of stage II flowers [Figs. 3A and 4A]); and (b) the appearance of 4.8 nmol/mg protein of 18:2/18:2 PG (the equivalent of 12.3% by weight of the total molecular species for PG), which was not detectable in membranes from stage II flowers (Fig. 5A) (18:2/18:2 is the second most abundant molecular species for PC and PE [Figs. 3 and 4]). During *in vitro* incubation the most abundant molecular species of PG, 16:0/18:2, again declined as did 16:0/18:3 (Fig. 5B). However, 16:0/16:0 PG, which increased during natural senescence (Fig. 5A), did not change during *in vitro* incubation (Fig. 5B). As well, 16:0/18:1 PG did not decline during *in vitro* incubation but did during natural senescence, and 18:2/18:2 PG was not formed during *in vitro* incubation but was formed during natural senescence (Fig. 5, A and B).

PI comprised only 7.3% of the total phospholipid in carnation microsomal membranes (Fig. 1B) and during senescence declined from 13.2 to 10 nmol/mg membrane protein (Fig. 1A). This decline reflects decreases on a nmol/mg protein basis in 16:0/18:1 PI, 16:0/18:2 PI, 18:0/18:2 PI, and 18:2/18:3 PI (Fig. 6A). During *in vitro* incubation, all of the molecular species of PI showed a decline on a nmol/mg protein basis (Fig. 6B).

For the purposes of comparison, the values for catabolism

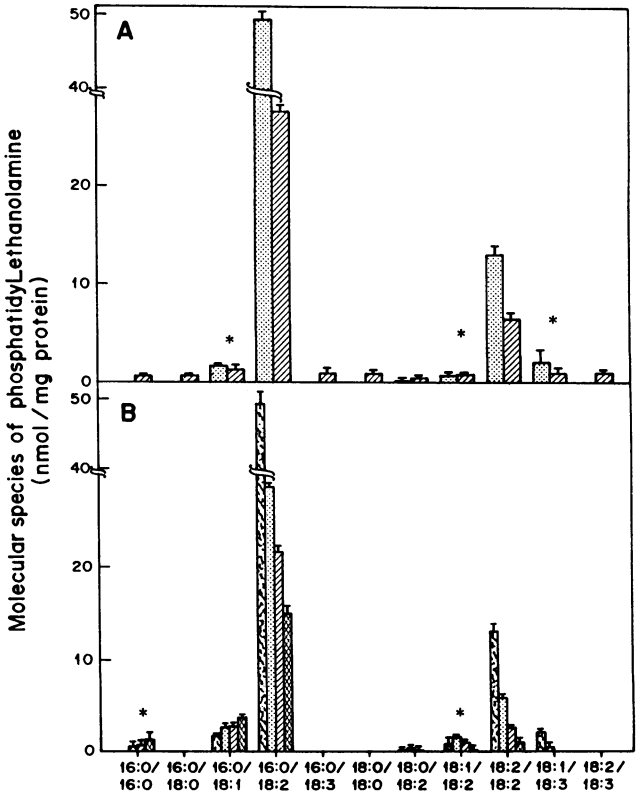

Figure 4. Molecular species composition of PE in microsomal membranes isolated from the petals of carnation flowers. A, Changes during natural senescence: left bars, young stage II flowers; right bars, senescent stage IV flowers. B, Changes during *in vitro* incubation of microsomal membranes from young stage II flowers. Bars left to right: nondialyzed control; dialyzed control at 0 h incubation; at 1.5 h incubation; at 3 h incubation. Means ± SE are shown for $n = 3$. Asterisks denote values that are not significantly different at $P = 0.05$.

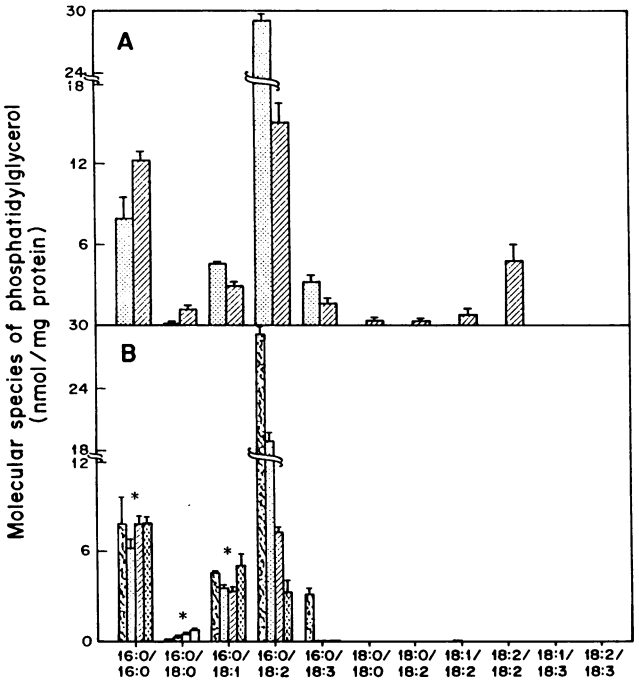

Figure 5. Molecular species composition of PG in microsomal membranes isolated from the petals of carnation flowers. A, Changes during natural senescence: left bars, young stage II flowers; right bars, senescent stage IV flowers. B, Changes during *in vitro* incubation of microsomal membranes from young stage II flowers. Bars left to right: nondialyzed control; dialyzed control at 0 h incubation; at 1.5 h incubation; at 3 h incubation. Means ± SE are shown for $n = 3$. Asterisks denote values that are not significantly different at $P = 0.05$.

of each molecular species during *in vitro* incubation of isolated membrane were normalized to PI, which was given an arbitrary value of 1 (Table I). In this comparison, if the head group is not an influencing factor, there should be no effect of head group on the catabolism of a particular molecular species. For some molecular species such as 16:0/18:2, 16:0/18:3, or 18:1/18:2, the degradation was different for various classes of phospholipid indicating that the head group influences susceptibility to catabolism. For example, 16:0/18:2 PG was degraded to a greater extent than 16:0/18:2 PE and 16:0/18:2 PI (Table I). It is noteworthy, however, that such differences were not evident for the more unsaturated molecular species, 18:2/18:2, 18:1/18:3, and 18:2/18:3. For these molecular species, the levels of degradation were the same irrespective of head group (Table I). The *in vitro* degradation data were also normalized to a single molecular species, 16:0/18:2, which was given an arbitrary value of 1, so that comparisons of the degradation of various molecular species within phospholipid classes could be made (Table II). These comparisons indicate that the acyl chain composition and the head group both influence phospholipid degradation. For example, although 16:0/18:2 PC was not preferred over 18:1/18:2 PC, 18:1/18:2 PI was preferred over 16:0/18:2 PI, and 18:1/18:2 PE was not degraded but 16:0/18:2 PE was degraded (Table II). The molecular species 18:1/18:3 and 18:2/18:3 were degraded to the same extent within phospholipid classes (Table II). The normalized data in Tables I and II are for absolute degradation values calculated as nmoles degraded per mg protein during dialysis and subsequent incubation of the isolated membranes. However, similar trends were obtained when values for percent degradation (*i.e.* percentage of initial amount degraded during dialysis and subsequent incubation) were normalized (data not shown).

Similar comparisons were made for the degradation data obtained during natural senescence. In Table III, degradation values have been normalized within each molecular species to PI, which has been given an arbitrary value of 1, to enable comparisons of molecular species breakdown among phospholipid classes. It is clear from these comparative data that the head group has a definite influence on the changes in phospholipid levels that occur during senescence. For example, for the 16:0/18:2 molecular species, PG declined to the greatest extent followed by PE, PC and, finally, PI, but this pattern was not consistent for all molecular species (Table III). It is noteworthy that in most cases the 18:1/18:3 and 18:2/18:3 molecular species did not decline during natural senescence (Table III), whereas during *in vitro* incubation

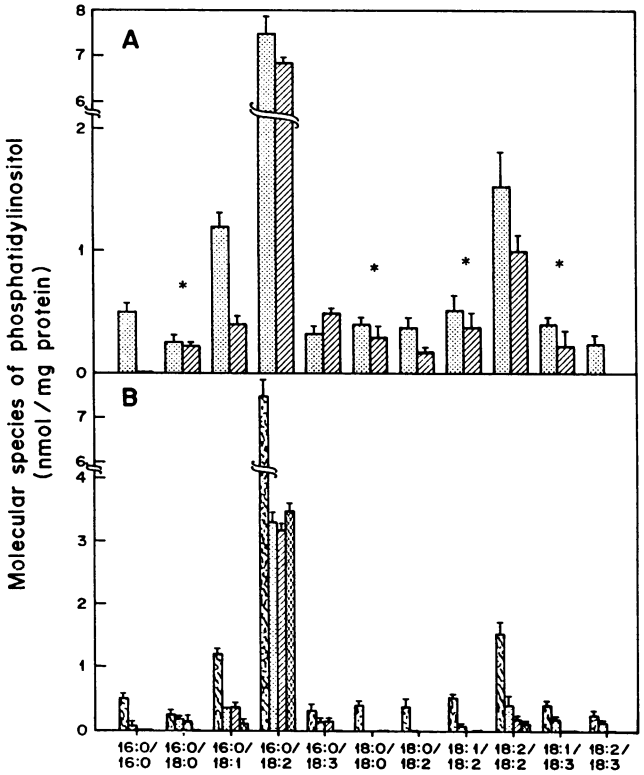

Figure 6. Molecular species composition of PI in microsomal membranes isolated from the petals of carnation flowers. A, Changes during natural senescence: left bars, young stage II flowers; right bars, senescent stage IV flowers. B, Changes during *in vitro* incubation of microsomal membranes from young stage II flowers. Bars left to right: nondialyzed control; dialyzed control at 0 h incubation; at 1.5 h incubation; at 3 h incubation. Means ± SE are shown for $n = 3$. Asterisks denote values that are not significantly different at $P = 0.05$.

Table I. *Effect of Differences in Head Group Composition on the Catabolism of Endogenous Phospholipid Molecular Species during in Vitro Incubation of Microsomal Membranes Isolated from Stage II Carnation Flowers*

Molecular Species	Relative Degrees of Catabolism[a]			
	PC[b]	PE	PG	PI
16:0/16:0	—[c]	ND	ND	1.0
16:0/18:0	ND[d]	—	ND	1.0
16:0/18:1	ND	ND	ND	1.0
16:0/18:2	1.1 (e)	1.2 (g)	1.7 (f)	1.0 (e)
16:0/18:3	0.8 (f)	ND	1.0 (e)	1.0 (e)
18:0/18:0	—	ND	—	1.0
18:0/18:2	ND	ND	—	1.0
18:1/18:2	0.5 (f)	ND	—	1.0 (e)
18:2/18:2	1.0 (e)	1.0 (e)	—	1.0 (e)
18:1/18:3	1.0 (e)	1.0 (e)	—	1.0 (e)
18:2/18:3	1.0 (e)	1.0 (e)	—	1.0 (e)

[a] Absolute degradation values (nmol/mg protein) obtained during dialysis and subsequent incubation were normalized to PI which was given an arbitrary value of 1 for each molecular species. For each molecular species, values followed by different letters in parentheses are significantly different at P = 0.05. [b] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol. [c] Not present. [d] No degradation.

Table II. *Effect of Differences in Molecular Species Composition on the Catabolism of Endogenous Phospholipid Classes during in Vitro Incubation of Microsomal Membranes Isolated from Stage II Carnation Flowers*

Reaction time was 3 h.

Molecular Species	Relative Degrees of Catabolism[a]			
	PC[b]	PE	PG	PI
16:0/16:0	—[c]	ND	ND	1.9 (e)
16:0/18:0	ND[d]	—	ND	1.9 (e)
16:0/18:1	ND	ND	ND	1.5 (f)
16:0/18:2	1.0 (e)	1.0 (e)	1.0 (e)	1.0 (g)
16:0/18:3	1.9 (f)	ND	1.2 (f)	1.9 (e)
18:0/18:0	—	ND	—	1.9 (e)
18:0/18:2	ND	ND	—	1.9 (e)
18:1/18:2	0.7 (e)	ND	—	1.9 (e)
18:2/18:2	1.9 (f)	1.7 (f)	—	1.4 (f)
18:1/18:3	2.4 (g)	2.1 (g)	—	1.9 (e)
18:2/18:3	2.4 (g)	2.1 (g)	—	1.9 (e)

[a] Absolute degradation values (nmol/mg protein) obtained during dialysis and subsequent incubation were normalized for each phospholipid class to 16:0/18:2 which was given an arbitrary value of 1. Within each column, values followed by different letters in parentheses are significantly different at P = 0.05. [b] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol. [c] Not present. [d] No degradation.

Table III. *Effect of Differences in Head Group Composition on the Catabolism of Endogenous Phospholipid Molecular Species of Microsomal Membranes during Senescence of the Carnation Flower (Stage II versus Stage IV)*

Molecular Species	Relative Degrees of Catabolism[a]			
	PC[b]	PE	PG	PI
16:0/16:0	—[c]	ND	ND	1.0
16:0/18:0	ND[d]	ND	ND	ND
16:0/18:1	1.9 (f)	—	0.9 (e)	1.0 (e)
16:0/18:2	3.0 (g)	4.9 (f)	5.3 (f)	1.0 (e)
16:0/18:3	ND	ND	1.0	ND
18:0/18:0	ND	ND	ND	ND
18:0/18:2	ND	ND	ND	1.0
18:1/18:2	1.0	ND	ND	ND
18:2/18:2	0.9 (e)	1.5 (f)	ND	1.0 (e)
18:1/18:3	ND	ND	—	ND
18:2/18:3	ND	ND	—	1.0

[a] Absolute degradation values (nmol/mg protein) were normalized to PI which was given an arbitrary value of 1 for each molecular species (except for 16:0/18:3 and 18:1/18:2 where PI did not decline and values were normalized to PG and PC, respectively). For each molecular species, values followed by different letters in parentheses are significantly different at P = 0.05. [b] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol. [c] Not present. [d] No decline (in some cases an increase).

these molecular species were extensively degraded (Table I). In Table IV, the values for degradation during natural senescence of the various molecular species have been normalized to 16:0/18:2, which was given an arbitrary value of 1 for each phospholipid class. This allows a comparison of the degradation of various molecular species within a phospholipid class, and it is clear from these data that the acyl chain composition of the phospholipids is a significant determinant of susceptibility to degradation. For example, for PI, the normalized degradation values range from 1 to 11 (Table IV). The normalized data in Tables III and IV are for absolute degradation values calculated in nmoles degraded per mg protein between stage II and stage IV of postharvest development, and they collectively indicate that phospholipid molecular species have distinctive patterns of degradation during senescence that appear to reflect an effect of both the head group and the acyl chains on susceptibility to enzymatic catabolism. Similar patterns were evident when values for percent degradation (*i.e.* percentage of the amount in stage II membranes degraded between stage II and stage IV) were normalized (data not shown).

DISCUSSION

During senescence of cut carnation flowers, there is a progressive decline in microsomal phospholipid. This is evident in part from the finding that the sterol:phospholipid ratio of microsomal membranes increases by about threefold as petal senescence advances (30). In addition, the ratio of phospholipid:total lipid in microsomal membranes from the petals decreases by 40% as the flowers age (9), and it is evident from the present study that the phospholipid:protein ratio of mi-

Table IV. *Effect of Differences in Molecular Species Composition on the Catabolism of Endogenous Phospholipid Classes of Microsomal Membranes during Senescence of the Carnation Flower (Stage II versus Stage IV)*

Molecular Species	Relative Degrees of Catabolism[a]			
	PC[b]	PE	PG	PI
16:0/16:0	—[c]	ND	ND	11.1 (e)
16:0/18:0	ND[d]	ND	ND	ND
16:0/18:1	2.7 (e)	—	0.8 (e)	4.2 (f)
16:0/18:2	1.0 (f)	1.0 (e)	1.0 (f)	1.0 (g)
16:0/18:3	ND	ND	1.1 (g)	ND
18:0/18:0	ND	ND	ND	ND
18:0/18:2	ND	ND	ND	6.0 (h)
18:1/18:2	3.2 (g)	ND	ND	ND
18:2/18:2	1.1 (f)	1.1 (f)	ND	3.8 (i)
18:1/18:3	ND	ND	—	ND
18:2/18:3	ND	ND	—	11.1 (e)

[a] Absolute degradation values (nmol/mg protein) were normalized for each phospholipid class to 16:0/18:2 which was given an arbitrary value of 1. Within each column, values followed by different letters in parentheses are significantly different at P = 0.05. [b] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol. [c] Not present. [d] No decline (in some cases an increase).

crosomes also decreases with age of the petals. Moreover, the four phospholipid classes in the microsomal membranes decline in parallel with advancing senescence such that the phospholipid composition based on head group analysis remains essentially unchanged.

The finding that there is no change in microsomal phospholipid composition suggests that all of the phospholipid classes are equally prone to catabolism. This was also evident when microsomal membranes isolated from young stage II flowers were incubated *in vitro*. Phospholipid levels declined by 65% over a 3 h period in keeping with an earlier finding that these membranes contain enzymes capable of catabolizing exogenous radiolabeled phospholipids (5, 23), yet there was no significant change in phospholipid head group composition. However, head group analyses do not take account of the fact that each phospholipid class contains a number of molecular species, and it is clear from the present study that certain molecular species of the phospholipid classes have quite different susceptibilities to enzymatic catabolism. This was evident during natural senescence and also during *in vitro* aging of isolated membranes. The comparative analyses of normalized degradation values indicate that the paired acyl chains as well as the head group of the membrane phospholipid are recognized by its catabolizing enzyme and are determinants of its susceptibility to enzymatic degradation. Indeed, although there are no significant changes in head group composition of the microsomal membranes during natural senescence of the petals or during *in vitro* aging of isolated membranes, there are marked changes in molecular species composition within each phospholipid class in both instances. Differences in the membrane species composition of the microsomal fractions for stage II and stage IV flowers could contribute to the changes in molecular species composition. However, the contention that this is due in part to different susceptibilities of the various molecular species to enzymatic catabolism is supported by the finding that *in vitro* incubation of isolated microsomes under conditions in which there was a net decline in total phospholipid also resulted in a change in molecular species composition for each phospholipid class.

This type of double recognition of phospholipid substrate by its catabolizing enzyme (*i.e.* recognition of the head group and the paired acyl chains) has been noted previously for human phospholipases. For example, phospholipase C from human monocytes shows a molecular species preference pattern for PC whereby 16:0/18:2 > 16:0/20:4 > 16:0/16:0 > 16:0/18:1, but the same enzyme exhibits no preference for any of the various molecular species of PI (2). Similarly, it has been reported that cytosolic phospholipase A_2 from human synovial fluid prefers 18:0/20:4 PC over 16:0/18:2 PC, but shows no preference between these two molecular species of PE (16).

The comparison of the changes in molecular species composition of microsomal membranes during natural senescence of the carnation flower and during *in vitro* aging (*i.e. in vitro* incubation of isolated membranes) has provided indirect evidence for the involvement of desaturases and possibly retailoring enzymes in membrane lipid breakdown during senescence. Many of the effects of natural senescence on microsomal membranes of carnation flowers can be simulated by *in vitro* aging of the membranes isolated from young tissue (9) but this was not so in respect of the phospholipid molecular species composition of the membranes. In particular, there was a large decline in polyunsaturated molecular species during *in vitro* aging, particularly those containing 18:2 and 18:3, that was less evident during natural senescence. It seems reasonable to attribute this to the fact that microsomal desaturases, which are able to convert 18:1 to 18:2 and 18:2 to 18:3 (12), retailoring enzymes and possibly synthetic enzymes are functional during natural senescence and continuously replenish molecular species containing 18:2 and 18:3. This is evident in part from the observations that certain lipid species found in the microsomes isolated from senescent stage IV flowers, such as 18:2/18:3 PE, 18:2/18:2 PG, and 18:0/18:0 PC, were not present in microsomes isolated from young stage II flowers. By contrast, the desaturases and retailoring enzymes would not be active in the *in vitro* incubation mixture because required cofactors were not present, and it is presumably for this reason that the phospholipid molecular species containing 18:2 and 18:3 were depleted during *in vitro* aging.

Desaturases and retailoring enzymes are both known to be associated with plant microsomal membranes (28). Retailoring involves the redistribution of fatty acids bound to a population of phospholipids to achieve different pairings of acyl chains (28). The identity of the enzymes mediating retailoring is not known (28), but the process is thought to be important in achieving rapid homeoviscous adaptation in membranes (6). It has been proposed previously that desaturase enzyme systems are activated when membrane lipid fluidity drops below a threshold level (28). This does not appear to be the basis for the increase in desaturase activity brought on by a reduction in temperature (31), but the relationship between membrane lipid fluidity and desaturase

activity is supported by several other lines of evidence. For example, it was shown in a recent study that the addition of 17:0/17:0 PC to rat thymocytes resulted in a pronounced rigidification of membranes as well as enhanced desaturase activity (32). It has been shown previously that there is a decrease in the lipid fluidity of the microsomal membranes from carnation flowers with advancing petal senescence (30), and it is conceivable that the indirect evidence for the involvement of desaturases in membrane senescence noted in the present study reflects desaturase activation attributable to this fluidity change.

Previous studies using exogenous radiolabeled phospholipid substrates have demonstrated that there is no change in phospholipase activity in microsomal membranes as the petals of carnation flowers senesce, yet there is a very marked decline in microsomal phospholipid levels (5, 9, 23). The results of the present study indicate that provision of particular molecular species of phospholipids with increased susceptibility to degradation may be the underlying basis for this enhanced phospholipid catabolism. The enzyme mediating this net catabolism of phospholipids has not been unequivocally identified, although recent studies with these membranes using exogenous radiolabeled PC and PI have indicated that the first step in phospholipid catabolism by these membranes is hydrolysis by phospholipase D to form phosphatidic acid (5, 23).

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