Plant Physiol. (1987) 85, 679–683
0032-0889/87/85/0679/05/$01.00/0

# Molecular Species Specificity of Phospholipid Breakdown in Microsomal Membranes of Senescing Carnation Flowers[1]

Received for publication January 13, 1987 and in revised form May 27, 1987

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## ABSTRACT

**During senescence of cut carnation flowers, there is extensive breakdown of microsomal phospholipid. This is attributable, at least in part, to lipolytic activity associated directly with the microsomal membranes. Evidence indicating that one or more of the lipid-degrading enzymes in these membranes preferentially degrade phospholipid molecular species containing two diunsaturated acyl chains or at least one polyunsaturated acyl chain has been obtained by using radiolabeled phosphatidylcholine substrates. 16:0*/16:0*, 16:0/18:2*, and 18:1*/18:1* phosphatidylcholine were degraded only minimally over a 3 hour period by microsomes isolated from senescing flowers. By contrast, [U-$^{14}$C]phosphatidylcholine, which comprises various molecular species including those containing polyunsaturated acyl chains, and 18:0/20:4* phosphatidylcholine were extensively degraded. Under identical conditions, but in the absence of added radiolabeled substrate, endogenous 18:2/18:2, 18:1/18:3, and 18:2/18:3 phosphatidylcholine were selectively depleted from the membranes. During natural senescence of the flowers, there was a sharp decline in microsomal 16:0/18:1 and 18:1/18:2 phosphatidylcholine, whereas molecular species containing two diunsaturated acyl chains or at least one polyunsaturated acyl chain remained unchanged or decreased only slightly. The data have been interpreted as indicating that provision of particular molecular species susceptible to lipase attack is a prerequisite to phospholipid catabolism in senescing membranes.**

Loss of membrane integrity during senescence is accompanied by a decline in phospholipid. This has been demonstrated for senescing carnation flowers (25), senescing rose petals (3), senescing flowers of *Ipomoea* (1), senescing *Tradescantia* petals (23), senescing cotyledons of cucumber and bean (6, 16) and ripening pepper fruit (15). It has been proposed that the decrease in phospholipids is the result of increased degradation, decreased synthesis, or possibly both (4, 23).

Phospholipid-degrading enzymes have been implicated as agents of lipid deterioration in senescing membranes (3, 4, 23). There appear to be three enzymes involved: phospholipase D, phosphatidic acid phosphatase, and lipolytic acyl hydrolase (8, 21). Borochov *et al.* (4) have reported an increase in phospholipase activity for senescing rose petals, but Suttle and Kende (23) found no change in phospholipase activity during senescence of *Tradescantia* petals. This suggests that in at least some senescing tissues the catabolism of membrane lipid is attributable to changes in the molecular organization of the bilayer that render the lipids more susceptible to attack by lipases.

In the present study, we describe evidence indicating that the phospholipid-degrading enzymes associated with microsomal membranes of senescing carnation flowers exhibit specificity for phospholipid molecular species containing two diunsaturated fatty acids or at least one polyunsaturated fatty acid. This finding is discussed in the context that provision of specific molecular species of phospholipids, which serve as preferred substrates for membrane-associated lipases, may regulate the progression and rate of phospholipid degradation in senescing membranes.

## MATERIALS AND METHODS

**Plant Material and Membrane Isolation.** Carnation flowers (*Dianthus caryophyllus* L. cv White Sim) were grown in a commercial greenhouse (Unsworth and Sons, Burlington, 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 maintained at 22°C under continuous illumination (240 ft-c) until they had reached specific stages of senescence, *viz.*, stage II, flowers that still possessed yellowish-tinted centers, but were fully expanded; stage III, flowers that were completely white in color but were not yet showing petal-inrolling; stage IV, senescent flowers showing petal-inrolling.

Microsomal membranes were isolated from stage II, III, and IV flowers in 10 mM Epps[3] (pH 8.5), as described previously (25) and washed once by resuspension in the same buffer and centrifugation at 131,000*g* for 1 h. The resulting pellet of membranes was resuspended in 3 ml 70 mM Epps (pH 8.5), and dialyzed at 4°C against 3 changes of 600 ml of 2 mM Epps (pH 8.5), for 15 h. After dialysis, the protein concentration was adjusted to 1 mg ml$^{-1}$ with 70 mM Epps (pH 8.5). Protein was measured as described by Bradford (5).

**Phospholipid Degradation.** The capability of isolated microsomal membranes to degrade various molecular species of phospholipids was determined by using radiolabeled substrates. An aliquot (1.8 ml) of washed, dialyzed membrane suspension (1 mg protein ml$^{-1}$) was vortexed for 3 min with 0.3 μCi of algal [U-$^{14}$C]phosphatidylcholine (1.9 Ci/mmol, New England Nuclear), 0.8 μCi of L-α-dipalmitoyl [dipalmitoyl-1-$^{14}$C]phosphatidylcholine (112 mCi/mmol, New England Nuclear), 0.8 μCi of L-α-1-palmitoyl-2-linoleoyl [linoleoyl-1-$^{14}$C]phosphatidylcholine (52 mCi/mmol, New England Nuclear), 0.8 μCi L-α-dioleoyl [dioleoyl-1-$^{14}$C]phosphatidylcholine (114 mCi/mmol, New England Nuclear), or 0.8 μCi L-α-1-stearoyl-2-arachidonyl-

[1] Supported by grants from the Natural Sciences and Engineering Research Council of Canada.

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[3] Abbreviations: Epps, *N*-2-(hydroxyethyl)piperazine-*N*'-3-propane sulfonic acid; Ches, 2(*N*-cyclohexylamino)ethane-sulfonic acid; MTBSTFA, *N*-methyl-*N*-(tert-butyldimethylsilyl)trifluoroacetatide; Tween 20, polyoxyethylene sorbitan monolaurate; U37184, (3,4-dichlorophenyl)hydrazone.

[arachidonyl-5,6,8,9,11,12,14,15-$^3$H(N)]phosphatidylcholine (180 Ci/mmol, New England Nuclear). The resulting suspension was divided into three equal samples, and each sample was diluted to 200 $\mu$g protein ml$^{-1}$ with 70 mM Epps (pH 8.5), in a 50 ml Pyrex centrifuge tube. One reaction mixture served as the zero-time control, and the others were incubated for 1.5 and 3 h, respectively, at 30°C in darkness to prevent any oxidation of lipid that might be facilitated by light. Phospholipase $A_2$ (3 units) (*Naja naja*) and U37184 (Upjohn Co., Kalamazoo, MI) were added to the reaction mixture as specified. U37184 was made up as a 10 mM stock solution in methanol, and 60 $\mu$l of the stock solution were added to the reaction mixture.

The reactions were terminated by lipid extraction (2), and the lipids were separated and identified by TLC (Silica gel 60 plates—E. Merck, Germany). The plates were first developed in chloroform:acetic acid:methanol:water (70:25:5:2, v/v/v/v) (11) and then developed again in petroleum ether:ethyl ether:acetic acid (70:30:1, v/v/v). The separated lipids were visualized by exposure to iodine vapor, scraped into 5 ml of liquifluor, and counted in a Beckman LS 7000 scintillation counter.

**Molecular Species Analysis.** Changes in the molecular species composition of endogenous phosphatidylcholine in microsomal membranes were measured during natural senescence and also during *in vitro* incubation over the same time-frame (*i.e.* zero-time, 1.5 h of incubation and 3 h of incubation) that was used for measuring the breakdown of exogenous radiolabeled phosphatidylcholine. For the *in vitro* reactions, 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 (2). For measurements of changes in molecular species composition during natural senescence, 5-ml samples of membrane suspension (1 mg protein ml$^{-1}$) were lipid-extracted directly (2). Molecular species of phosphatidylcholine were isolated from the lipid extracts and converted to the corresponding diacylglycerols using phospholipase C (Boehringer Manheim), derivatized with MTBSTFA (Pierce Chemical Co.) to form *t*-BDMS derivatives and analyzsed by GLC as described previously (11). The chromatography was performed using a Shimadzu Gas Chromatograph 9A equipped with a 15 m × 0.25 mm (i.d.) open tubular capillary glass column coated with SP2330 (Supelco). The column, injector, and detector temperatures were 248, 280, and 280°C, respectively, and the instrument was operated isothermally at a split ratio of 20:1.

The separated molecular species were identified by comparison with the known separation pattern of *t*-BDMS-diacylglycerol derivatives of purified soybean phosphatidylcholine (18). Confirmation of identity was obtained by comparing the fatty acid composition of the separated molecular species, as determined from their assigned identities, with the measured fatty acid composition of phosphatidylcholine. Fatty acid methylation was carried out according to Morrison and Smith (17). The methyl esters were separated isothermally with a split ratio of 40:1 on the same column that was used for molecular species separation. The column, injector, and detector temperatures were 200, 250, and 250°C, respectively. The separated methyl esters were identified by co-chromatography with rapeseed oil standards.

## RESULTS

**Degradation of Radiolabeled Phosphatidylcholine.** The degradation of [U-$^{14}$C]phosphatidylcholine by microsomal membranes from the petals of stage III carnation flowers is illustrated in Table I. Five discrete breakdown products were discernible after 3 h of incubation: phosphatidic acid, diacylglycerol, free fatty acids, fatty acid oxidation products, and an unknown component that was not identified. Each of phosphatidic acid, diacylglycerols, and free fatty acids were identified by co-chromatography with authentic standards. Fatty acid oxidation products were identified by co-chromatography with the products of the lipoxygenase reaction. The reaction mixture for this purpose contained linoleate (1.6 mM), 0.5% Tween, 20 and an excess (0.25 mg ml$^{-1}$) of soybean lipoxygenase (Sigma) in 10 ml of 50 mM Ches buffer (pH 8.6). After 20 min, the lipid hydroperoxides were extracted from diethyl ether and used for co-chromatography.

Table I. *Degradation of [U-$^{14}$C]phosphatidylcholine by Microsomal Membranes from the Petals of Stage III Carnation Flowers*

Incubation times (h) are specified. Data are shown for one of three separate experiments all showing the same trend.

| | Total Radioactivity | | |
|---|---|---|---|
| | 0 h | 1.5 h | 3 h |
| | | % | |
| Phosphatidylcholine | 87.6 | 54.7 | 43.7 |
| Unknown | 2.2 | 3.7 | 5.7 |
| Phosphatidic Acid | 5.8 | 13.6 | 13.6 |
| Fatty acid oxidation products | 2.7 | 20.2 | 26.1 |
| Diacylglycerol | <1 | 4.0 | 4.9 |
| Free fatty acids | <1 | 4.0 | 6.0 |

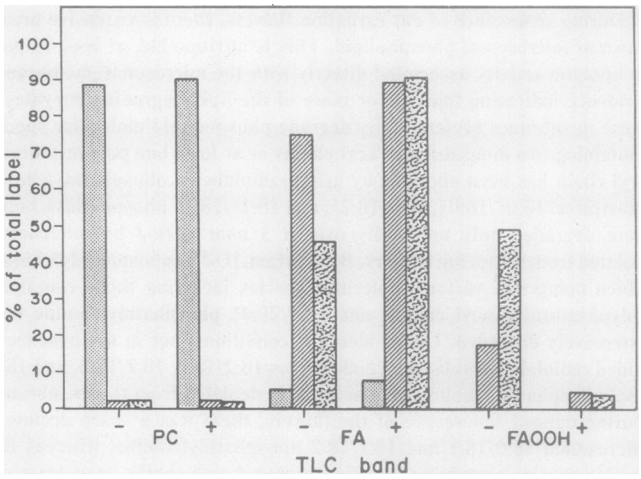


FIG. 1. Effect of the lipoxygenase inhibitor, U37184 (0.2 mM), on the metabolism of radiolabeled linoleic acid by microsomal membranes from the petals of stage III carnation flowers. Radiolabeled linoleic acid was generated by adding 16:0/18:2* phosphatidylcholine and phospholipase $A_2$ (3 units) to the reaction mixture. PC, phosphatidylcholine; FA, free fatty acid; FAOOH, fatty acid oxidation products; TLC band, band scraped from the TLC plate. Values are from one of three separate experiments all showing the same trend. Stippled bars, 0 h of incubation; hatched bars, 1.5 h of incubation; mottled bars, 3 h of incubation. (−), No inhibitor; (+), inhibitor added.

Lipoxygenase-like activity is associated with microsomal membranes from carnation flowers (13), and the fatty acid oxidation products derived from [U-$^{14}$C]phosphatidylcholine were also identified indirectly by following the metabolism of radiolabeled linoleic acid in the presence and absence of U37814, a specific inhibitor of lipoxygenase. Radiolabeled linoleic acid was generated by adding 16:0/18:2* phosphatidylcholine and exogenous phospholipase $A_2$ to the microsomal membranes. At time zero, a small amount of linoleic acid was detectable (Fig. 1). Within 1.5 h in the absence of the inhibitor, the level of free linoleic acid had increased to >70% of the total label, and by 3 h had decreased to ≅45% of the total label. The decrease in linoleic acid between 1.5 and 3 h could be accounted for by an increase in fatty acid oxidation products (Fig. 1). There were no detectable fatty acid

oxidation products at time zero but the levels increased to ≅18% of the total label within 1.5 h and to ≅50% of the total label by 3 h. In the presence of the inhibitor, fatty acid oxidation products accounted for ≅5% of the total label after 3 h, and the breakdown of 16:0/18:2* phosphatidylcholine was matched by a corresponding increase in linoleic acid (Fig. 1).

**Molecular Species Specificity of Lipid Degrading Enzymes.** It is apparent from the data in Table I that the rate at which [U-$^{14}$C]phosphatidylcholine was converted to radiolabeled degradation products decreased over the 3-h reaction period. During the first 90 min, 46.3 ± 1.1% of the [U-$^{14}$C]phosphatidylcholine was degraded, whereas from 1.5 h to 3 h there was degradation of only 16.8 ± 2.9% of the remaining substrate. This apparent decline in lipase activity suggests a depletion of specific reactive components of the substrate, which could occur if one or more of the enzymes involved were molecular species-specific. [U-$^{14}$C] phosphatidylcholine contains a variety of fatty acids linked to glycerol in various combinations to form distinguishable molecular species (18), and only some of these may be substrates for the lipid-degrading enzymes of senescing microsomal membranes.

This possibility was examined by using four radiolabeled phospholipids, 16:0*/16:0* phosphatidylcholine, 16:0/18:2* phosphatidylcholine, 18:1*/18:1* phosphatidylcholine, and 18:0/20:4* phosphatidylcholine in addition to [U-$^{14}$C]phosphatidylcholine as substrates for the lipases of microsomal membranes from the petals of stage III flowers. Whereas 59% of the [U-$^{14}$C] phosphatidylcholine and 53% of the 18:0/20:4* phosphatidylcholine were degraded within 3 h, only 9% of the 16:0*/16:0* phosphatidylcholine, 12% of the 16:0/18:2* phosphatidylcholine and 15% of the 18:1*/18:1* phosphatidylcholine were degraded over the same period (Fig. 2). These observations indicate that disaturated molecular species, diunsaturated molecular species containing monounsaturated fatty acids, and molecular species containing one saturated acyl chain and one diunsaturated acyl chain are not preferred substrates for the microsomal enzymes. It can be inferred from the data obtained with [U-$^{14}$C]phosphatidylcholine that the lipid degrading enzymes of these membranes readily degrade more perturbed molecular species of phosphatidylcholine such as those containing two diunsaturated acyl chains or at least one polyunsaturated acyl chain. This is supported by the finding that 18:0/20:4* phosphatidylcholine is extensively catabolized. This same specificity was also observed for membranes isolated from the petals of more senescent stage IV flowers (Fig. 3) and when the reactions were carried out at pH 7.3 (data not shown), a pH at which the lipases are active, but the lipoxygenase-like activity associated with these membranes is reduced (7). It was not possible to measure the molecular species composition of the algal [U-$^{14}$C]phosphatidylcholine used as a substrate because of the large amounts of material required for these analyses. However, it has been previously reported for the green algae, *Dunaliella salina*, cultured at 12°C that about 53% of the molecular species of phosphatidylcholine contain two diunsaturated acyl chains or at least one polyunsaturated acyl chain (11). This is in reasonable agreement with the finding in the present study that >50% of the algal [U-$^{14}$C] phosphatidylcholine was degraded over a 3 h period (Figs. 2 and 3).

**Changes in the Molecular Species Composition of Endogenous Phosphatidylcholine.** The molecular species composition of endogenous microsomal phosphatidylcholine was examined during the 3-h incubation period used to measure the degradation of exogenous radiolabeled phosphatidylcholine substrates. Phosphatidylcholine comprises ≅32% of the total phospholipid in these membranes and was found to contain the following molecular species: 16:0/18:1, 16:0/18:2$^{\Delta 9,12}$; 16:0/18:3$^{\Delta 9,12,15}$; 18:0/18:0; 18:0/18:2$^{\Delta 9,12}$; 18:1/18:2$^{\Delta 9,12}$; 18:2$^{\Delta 9,12}$/18:2$^{\Delta 9,12}$; 18:1/18:3$^{\Delta 9,12,15}$; and 18:2$^{\Delta 9,12}$/18:3$^{\Delta 9,12,15}$. During dialysis of microsomal membranes isolated from stage II flowers and a subsequent 3 h incubation period, there were changes in the molecular species composition of this phospholipid. In particular, 18:2/18:2, 18:1/18:3, and 18:2/18:3 phosphatidylcholine declined dramatically during dialysis of the membranes and the ensuing incubation period (Fig. 4). In terms of total lipid, 18:2/18:2 declined from 15.6 nmol/mg total lipid to 2 nmol/mg total lipid; 18:1/18:3 declined from 1.6 nmol/mg total lipid to undetectable levels; and 18:2/18:3 declined from 1.5 nmol/mg total lipid to undetectable levels. In fact, by the end of the dialysis period, 18:1/18:3 and 18:2/18:3 phosphatidylcholine were essentially depleted, and 18:2/18:2 phosphatidylcholine had decreased by ≅50% (Fig. 4). The molecular species 16:0/18:1 phosphatidylcholine and 16:0/18:2 phosphatidylcholine increased on a percentage basis reflective of compensatory increases due to the loss

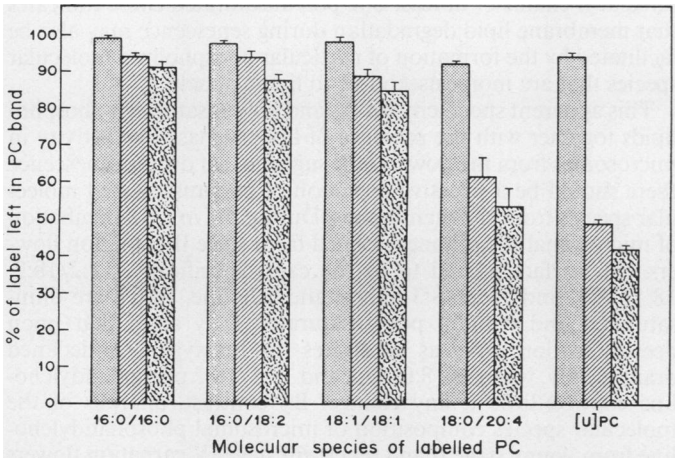


FIG. 2. Degradation of radiolabeled molecular species of phosphatidylcholine by microsomal membranes from the petals of stage III carnation flowers. The substrates were: 16:0*/16:0* phosphatidylcholine; 16:0/18:2* phosphatidylcholine; 18:1*/18:1* phosphatidylcholine; 18:0/20:4* phosphatidylcholine and [U-$^{14}$C]phosphatidylcholine. Means ± SE are shown; $n$ = three separate experiments. Stippled bars, 0 h of incubation; hatched bars, 1.5 h of incubation; mottled bars, 3 h of incubation.

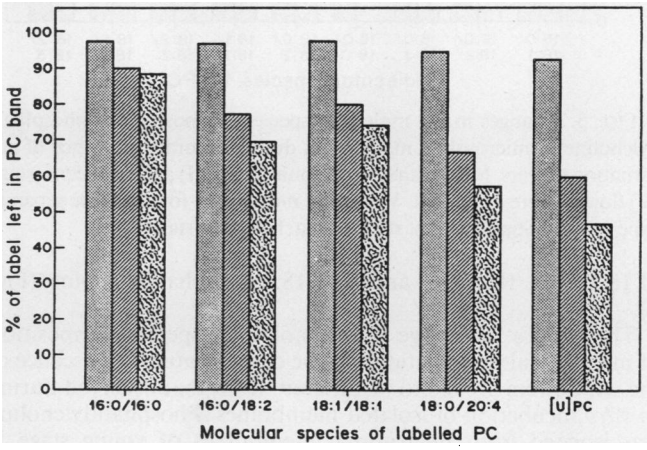


FIG. 3. Degradation of radiolabeled molecular species of phosphatidylcholine by microsomal membranes from the petals of stage IV carnation flowers. The substrates were 16:0*/16:0* phosphatidylcholine; 16:0/18:2* phosphatidylcholine; 18:1*/18:1* phosphatidylcholine; 18:0/20:4* phosphatidylcholine and [U-$^{14}$C]phosphatidylcholine. Data for [U-$^{14}$C]phosphatidylcholine are from one experiment. Data for the other substrates are averages of the values obtained in two separate experiments which differed by ≤3.3%. Stippled bars, 0 h of incubation; hatched bars, 1.5 h of incubation; mottled bars, 3 h of incubation.

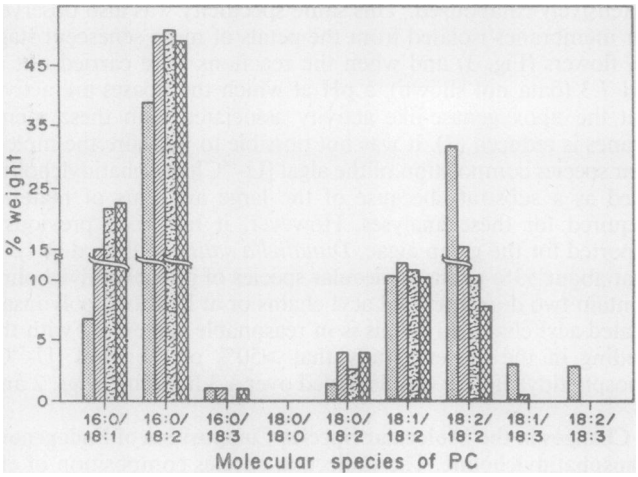


FIG. 4. Changes in the molecular species composition of phosphatidylcholine during *in vitro* aging of microsomal membranes isolated from the petals of stage II carnation flowers. Values shown are from one of three separate experiments all showing the same trends. Stippled bars, nondialyzed control; hatched bars, dialyzed control at 0 h of incubation; mottled bars, 1.5 h of incubation after dialysis; cross-hatched bars, 3 h of incubation after dialysis.

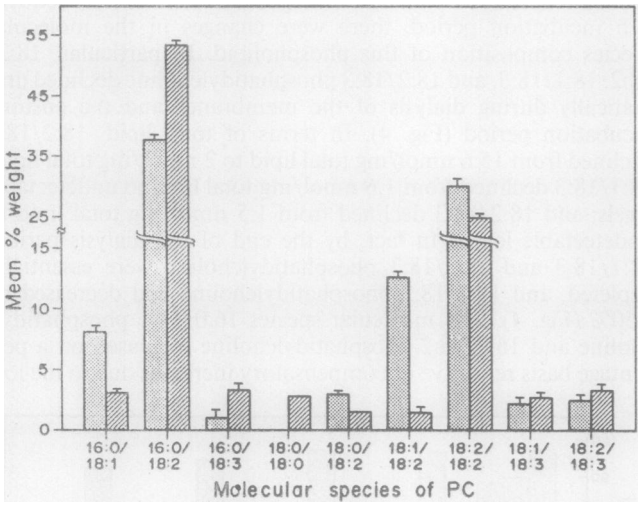


FIG. 5. Changes in the molecular species composition of phosphatidylcholine in microsomal membranes during natural senescence of cut carnation flowers. Membranes from young (stage II) and senescent (stage IV) flowers were examined. Values are means ± SE for $n$ = three separate experiments. Stippled bars, stage II; hatched bars, stage IV.

of 18:2/18:2, 18:1/18:3 and 18:2/18:3 phosphatidylcholine (Fig. 4).

The pattern of change in the molecular species composition of microsomal phosphatidylcholine during natural senescence of the cut flowers proved to be different from that observed during *in vitro* incubation of isolated membranes. Phosphatidylcholine was isolated from microsomal membranes of young stage II flowers and senescent stage IV flowers. During this period, the molecular species, 16:0/18:1 and 18:1/18:2, declined on a percentage basis, whereas levels of 18:1/18:3 and 18:2/18:3, molecular species that were depleted during dialysis and *in vitro* incubation, remained unchanged, and 18:2/18:2 and 18:0/18:2 declined only slightly (Fig. 5). In terms of total lipid, 16:0/18:1 declined from 3.9 nmol/mg total lipid at stage II to 0.94 nmol/mg total lipid at stage IV, and 18:1/18:2 declined from 6.2 nmol/mg total lipid at stage II to 0.43 nmol/mg total lipid at stage IV. The molecular species, 18:0/18:0 phosphatidylcholine, was not detected in membranes from stage II flowers but was present in membranes from stage IV flowers (Fig. 5). Similar changes were observed when total phosphatidylcholine, as distinct from microsomal phosphatidylcholine, was examined (data not shown). This indicates that the changes observed for microsomal phosphatidylcholine were not simply attributable to differences with age in the composition of the microsomal membrane fraction.

## DISCUSSION

Three enzymes, phospholipase D, phosphatidic acid phosphatase, and lipolytic acyl hydrolase, are thought to mediate the breakdown of membrane phospholipids in plant tissues (8, 21). There are both cytosolic and membranous forms of these enzymes (8, 20, 21, 26, 27). The former are thought to originate from autophagic organelles (8) and are apparently released during homogenization of the tissue. The products of phospholipase D, phosphatidic acid phosphatase and lipolytic acyl hydrolase are phosphatidic acid, diacylglycerol, and free fatty acids, respectively. The finding in the present study that these three lipid components are among the radiolabeled products formed during the degradation of [U-$^{14}$C]phosphatidylcholine by microsomal membranes suggests that the radiolabeled substrate is being metabolized by these enzymes. That the lipid-degrading activity is a property of the membranes and not attributable to cytosolic contaminants adhering to the isolated membranes or entrapped within them is supported by the fact that the membranes were washed before being used. As well, it has been previously reported (21) that microsomal phospholipase D, phosphatidic acid phosphatase, and lipolytic acyl hydrolase are enriched in the particulate fraction remaining after treatment of the membranes with 0.2% (v/v) Triton X-100.

The acyl chains of phospholipids are known to be an important determinant of turnover rates. In general, phospholipids containing short, unsaturated chains are more susceptible to attack by phospholipases and turn over faster than molecular species with long saturated chains (22). The acyl transferases that mediate repositioning of fatty acids in response to specific stimuli also have a marked specificity for polyunsaturated fatty acids (9). The finding in the present study that the lipid-degrading enzymes of microsomal membranes from aging carnation petals preferentially degrade phospholipid substrates containing two diunsaturated acyl chains or at least one polyunsaturated chain indicates that membrane lipid degradation during senescence may also be facilitated by the formation of particular phospholipid molecular species that are more susceptible to lipase attack.

This apparent specificity for di- and polyunsaturated phospholipids together with the presence of lipoxygenase-like activity in microsomes from the flowers (13) suggests that during senescence there should be a selective depletion of polyunsaturated molecular species from the membranes. During the *in vitro* incubation of microsomal membranes isolated from stage II carnation flowers, this in fact proved to be the case. Specifically, 18:2/18:2, 18:1/18:3, and 18:2/18:3 phosphatidylcholine, which are diunsaturated and contain polyunsaturated fatty acids that upon deesterification serve as substrates for lipoxygenase, declined dramatically, whereas 18:0/18:2 and 18:1/18:2 phosphatidylcholine showed little if any change. By contrast, analysis of the molecular species composition of microsomal phosphatidylcholine from young stage II and senescent stage IV carnation flowers indicated that molecular species containing two polyunsaturated fatty acids were not selectively degraded during natural senescence. Rather, 16:0/18:1 and 18:1/18:2 phosphatidylcholine decreased, whereas only small amounts of 18:2/18:2 and 18:0/18:2 were lost. These data are in agreement with corresponding measurements of total fatty acid composition for the same membranes, which have shown that oleic acid (18:1) declines during

natural senescence whereas linoleic (18:2) and linolenic (18:3) acids are depleted during *in vitro* aging of isolated membranes (7).

Molecular species analysis is more informative than fatty acid analysis in that it not only reveals changes in overall acyl chain composition but also fluctuations in constituent pairing. This is important since the acyl chain composition as well as the arrangement of the acyl chains within the molecular species determine the properties of the lipid bilayer (12). During *in vitro* incubation of isolated membranes, the molecular species containing two di- or polyunsaturated chains were preferentially degraded. This is probably also occurring during natural senescence, but would tend to be masked by the conversion of other molecular species such as 16:0/18:1 and 18:1/18:2 phosphatidylcholine into polyunsaturated molecular species through the action of desaturases and retailoring enzymes. This contention is supported by the finding that 16:0/18:1 and 18:1/18:2 phosphatidylcholine decline during natural senescence. It is also noteworthy that microsomal desaturases appear to be activated when bilayer fluidity is decreased (10, 14, 19, 24). Thus, the progressive decrease in microsomal membrane fluidity that accompanies senescence of cut carnation flowers (25) is likely to maintain the desaturases in an active state and thereby facilitate the formation of polyunsaturated molecular species prone to lipolytic hydrolysis.

*Acknowledgment*—The authors are indebted to James Chambers for excellent technical assistance.

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