JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. **34**, 590–600 (1999)

Fast Atom Bombardment Tandem Mass Spectrometric Analysis of Phospholipids in *Drosophila melanogaster*

S. Gamo,[1]* A. Kawabe,[1] H. Kohara,[2] H. Yamaguchi,[1]† Y. Tanaka[1] and S. Yagi[3]

[1] Department of Life Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai 599-8531, Japan
[2] Department of Material Sciences, Osaka Prefective University, 1-1 Gakuen-cho, Sakai 599-8531, Japan
[3] Department of Genetics, Tokushima University, Tokushima 770-8041, Japan

Direct determination of the phospholipid components in adult *Drosophila melanogaster* was carried out by using fast atom bombardment tandem mass spectrometry (FAB-MS/MS) of both the positive and negative ions. Approximately 50 molecular species were detected, including phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI). Eight PE, one PC and three PS molecular species were identified. Some variations with age and a few differences among the *D. melanogaster* strains in the PE and PC molecular species were found. There was a difference in the fatty acid structure of a 741 Da PE molecular species between the wild-types and a mutant strain *(Eth^{AR}201)* which requires a higher concentration of diethylether for anesthesia than the wild-types; in the mutant *sn*-1-oleoyl-2-linoleoyl (18 : 1/18 : 2) but in the wild-types *sn*-1-linoleoyl-2-oleoyl (18 : 2/18 : 1) were speculated. This suggests that this technique will be useful for the screening of phospholipid molecular species mutation.

KEYWORDS: glycerophospholipids; fast atom bombardment tandem mass spectrometry; mutant of phosphatidy lethanolamine species; aging effects; *Drosophila melanogaster*

INTRODUCTION

In living organisms, more than 130 phospholipid molecular species have been identified by mass spectrometric analysis.[1,2] Phospholipids are major components of biological membranes and play an important role in membrane functions such as membrane fluidity, permeability, transport, energy exchange and signal transduction. However, the biological activities of only a few species have been determined. The platelet activating factor (1-*O*-alkyl-2-acetyl-*sn*-3-glycerophosphocholine) mediates inflammatory reactions.[3,4] Phosphatidylinositol 4,5-bisphosphate is degraded to inositol phosphates and diacylglycerol (DG), which serve as intracellular messenger molecules.[5] Arachidonic acid released from intracellular phospholipids is a precursor of eicosanoids, prostaglandins, prostacyclins, leukotrienes and thromboxanes.[6,7] One convenient method, fast atom bombardment tandem mass spectrometry (FAB-MS/MS), allows phospholipid molecular species to be analyzed directly without prior derivatization and separation of crude samples in bacteria[8] and other biological materials.[9,10]

In *Drosophila melanogaster*, many molecular genetic studies have been carried out but few experiments on phospholipids have been performed. Our interest in *D. melanogaster* phospholipid species stems from their usefulness for screening mutants and for mutation analysis. In our previous research on the determination of the phosphatidylethanolamine (PE) and phosphatidylcholine (PC) molecular species of *tert*-butyl dimethylsilyl (TBDMS) derivatives, we found some strain-dependent differences in the PE molecular species between *D. melanogaster* ether-resistant *(Eth^{AR} 201)* and ether-sensitive strains by using gas–liquid chromatographic and mass spectrometric analyses (GC/MS),[11] not by direct analysis as is now used. The ether-resistant strain needs a higher concentration and the ether-sensitive strain requires a lower concentration of diethylether to lead to anesthesia than wild-type strains. Several investigators have shown the differences in some phospholipid classes in the visual mutants of adult flies[12,13] and larvae[14] by the use of thin-layer chromatography (TLC). Total PE was determined by TLC in the analysis of bang-sensitive mutation (*easily shocked*) in ethanolamine kinase gene.[15]

In this work, *D. melanogaster* phospholipid molecular species were analyzed by using FAB-MS/MS, which has the advantage of direct analysis of crude lipid samples and can reveal molecular species that have speculated fatty acyls. This method was applied to age effects and strain-dependent differences. The findings indicate that it facilitates the screening of mutants in phospholipid molecular species.

EXPERIMENTAL

Materials

In *Drosophila melanogaster* two wild-type strains, *Canton-S* and *Oregon-R*,[16] and *Eth^{AR}201* (spontaneously

* *Correspondence to*: S. Gamo, Department of Life Sciences, Osaka Prefecture University, 1-1 Gakuen-Cho, Sakai 599-8531, Japan.
E-mail: gamo@el.cias.osakafu-u.ac.jp
† *Present address*: Proctor and Gamble Far East, Inc., Shiga Technical Center, Shiga, Japan.

CCC 1076–5174/99/060590–11 $17.50

Received 29 June 1998
Accepted 23 February 1999

ether-resistant mutant, previously called *Eth-29*)[16,17] were used. The flies were cultured in our standard medium at 25 °C.[17]

Sample preparation

Total lipids were extracted from adult flies by the modified method of Bligh and Dyer.[18] The phospholipid classes were fractionated by using two-dimensional (2D) TLC. The first developing solvent was chloroform–methanol–water (65 : 35 : 5, v/v) and the second solvent was chloroform–acetone–methanol–acetic acid–water (10 : 4 : 2 : 2 : 1, v/v). Phospholipids were detected by using Dittmer–Lester reagent.[19] Phospholipid classes were subsequently separated by preparative TLC with the developing solvent chloroform–methanol–water (65 : 25 : 4, v/v). Most of the chemical reagents were obtained from Nacalai Tesque (Nara, Japan). Aliquots of phospholipid fractions and total lipids were subjected to mass spectrometric analysis. Four samples were prepared and evaluated by Student's *t*-test for comparison of mass profiles.

Mass spectrometry

Mass spectra were obtained with a Finnigan TSQ-70 triple-stage quadrupole mass spectrometer. Xenon was used as the particle source for FAB. A FAB gun (Ion Tech) was operated at 2 mA with an acceleration of 8 keV. Argon at a pressure of 0.5–1 mTorr (1 Torr =

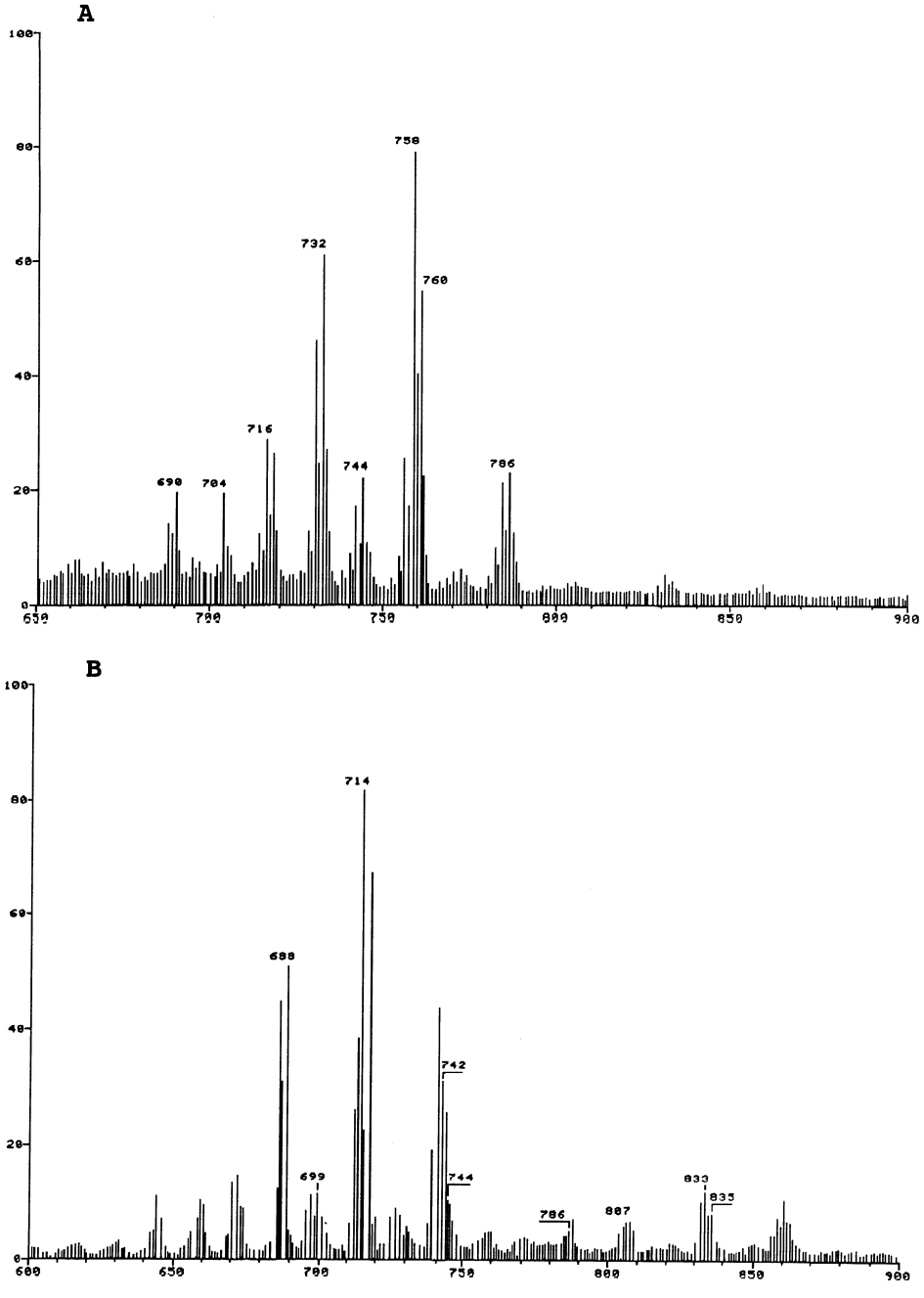

Figure 1. FAB-MS analysis of *D. melanogaster* phospholipids: (A) positive ion detection; (B) negative ion detection in total lipids from 5-day-old *Canton-S*. The prominent ions in the positive and negative ion FAB mass spectra are PC and PE, respectively.

133.3 Pa) was used and the collision energy offset was 15–20 eV. These conditions ensured multiple collisions between the target gas and the ion exiting the first quadrupole mass spectrometer. Approximately 1–2 μl of glycerol–3-nitrobenzyl alcohol (1 : 1, v/v) was used as the matrix for FAB. The total lipids and phospholipid fractions from the preparative TLC for FAB analysis were dissolved in chloroform at typical concentrations of 4 and 2 mg/ml^{-1}, respectively. A 1 μl aliquot was added to the matrix which was already in place on the probe tip, and allowed to evaporate for 1 min at room temperature before analysis.

RESULTS AND DISCUSSION

D. melanogaster phospholipid classes

Phospholipids in the total lipids of *D. melanogaster* adult flies were analyzed by positive and negative FAB-MS. More than 20 peaks appear in the positive FAB mass spectrum in the molecular mass region of the glycerophospholipids in Fig. 1(A). Mostly the prominent ions correspond to $[M + H]^+$ derived from individual molecular species of PC (see also Fig. 4), and PE (see also Fig. 2). In the negative ion FAB-MS (Fig. 1(B)), mostly the molecular species of PE correspond to $[M - H]^-$. Since each phospholipid class has different ionization efficiencies in the positive and negative ion modes, apparent ion abundances in the normal scanning do not reflect the actual abundances of phospholipid classes. Figure 1(A) (positive ion mode) shows high ion abundances for the quaternary ammonium cation of PC, while Fig. 1(B) (negative ion mode) shows high ion abundances for $[M - H]^-$ ions releasing a proton from the phosphate group of PE. This is because the ionization efficiencies in both polarities are very dependent on the chemical structures of the polar head groups of individual phospholipid classes.

In order to examine the molecular species of each phospholipid class, the total lipids were fractionated into seven classes by 2D-TLC. They were PE, PC, an unidentified phospholipid at a position close to phosphatidylglycerol, phosphatidylserine (PS), phosphatidylinositol (PI), lysophosphatidyl ethanolamine and lysophosphatidylcholine, in order of the apparent amount of individual phospholipid classes on TLC (data not shown). Based on the estimation of phosphate in glycerophospholipids, PE was the most (37–38%) and PC was the second most abundant (15–17%) in *D. melanogaster*.[11] PE, PC, PS and PI spots were collected by preparative TLC and applied to FAB-MS and FAB-MS/MS.

PE molecular species in *D. melanogaster*

FAB-MS/MS using a neutral-loss scan of *m/z* 141, corresponding to the phosphoethanolamine polar head group ($H_2PO_4CH_2CH_2NH_2$) from the $[M + H]^+$ ions, has been suggested for use in identifying the positive ions arising from PE molecular species.[8] A low-energy collision-induced decomposition (CID) reaction occurs to cause the loss of *m/z* 141 from the phosphoethanolamine head group. These spectra of the *D. melanogaster* total lipids extracts (Fig. 2(A)) and PE fractions (Fig. 2(B)) appeared to be almost the same. This indicates that the use of neutral loss scanning essentially obviated the need for lipid class separation by TLC prior to FAB-MS. At least 10 diacyl PE molecular species were detected and we were able to determine the total number of carbon atoms and the total number of double bonds in the two fatty acid groups: $[M + H]^+$ at *m/z* 662 (30 : 1), 688 (32 : 2), 690 (32 : 1), 714 (34 : 3), 716 (34 : 2), 718 (34 : 1), 740 (36 : 4), 742 (36 : 3), 744 (36 : 2) and 746 (36 : 1) in 5-day-old flies. Previously we have detected 12 molecular species of PE TBDMS esters in 1-day-old flies by using GC and GC/MS:[11] 30 : 1, 30 : 0, 32 : 2, 32 : 1, 32 : 0, 34 : 3, 34 : 2, 34 : 1, 36 : 5, 36 : 4, 36 : 3 and 36 : 2.

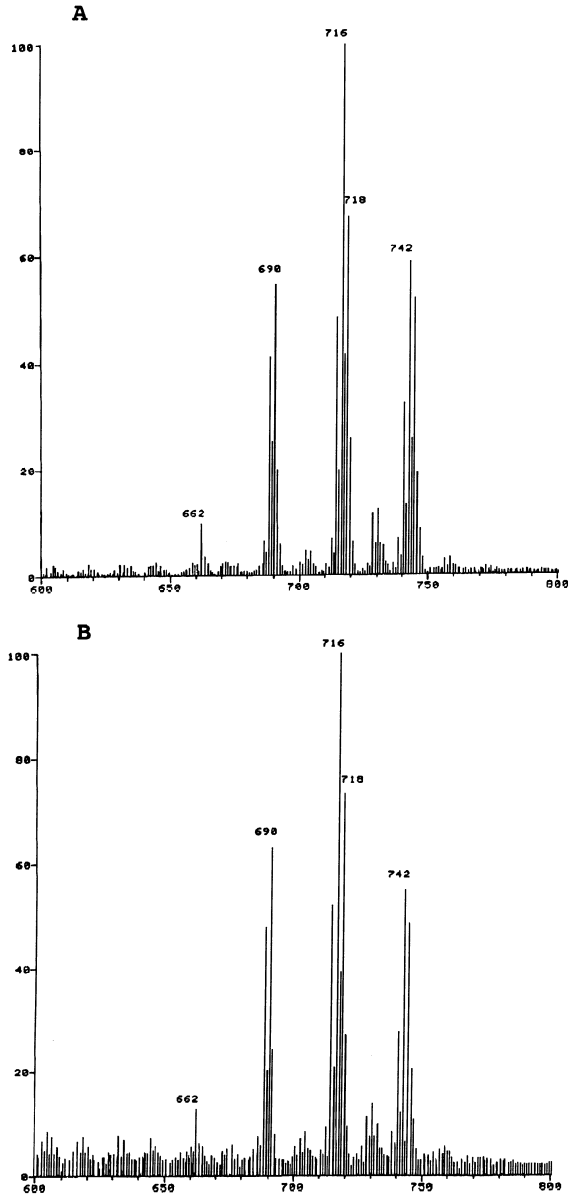

Figure 2. Neutral-loss scan for PE (loss of *m/z* 141) in *D. melanogaster*: (A) in total lipids; (B) purified PE fraction by TLC from 5-day-old *Canton-S*. The percentage relative ion abundances of 10 PE species were not significantly different between total lipid extracts and PE fractions when Student's *t*-test was applied.

The major PE molecular species were analyzed by using a product ion scan to dissociate the negative ions and to examine the two fatty acyl groups. The abundance of the ion derived from the *sn*-2 substituent is, in many cases, greater than that produced by the *sn*-1 fatty acid.[8,9] However, the positions of the fatty acyl substitution for PEs are speculation based predominantly on the results reported for PCs.[20,21] This rule apparently does hold for phospholipid-containing fatty acids with fewer than three points of unsaturation and with chain lengths differing by less than 10 carbons from each other.[21] The fatty acid composition of *D. melanogaster* phospholipids contained the shortest 14 carbons and the longest 18 carbons within three points of unsaturation.[11] Among PE molecular species, isobaric $[M-H]^-$ ion at *m/z* 688 consists mostly of 16 : 0/16 : 1 (*m/z* 255 and 253) and very little 14 : 0/18 : 1 (*m/z* 227 and 281) in Fig. 3(A). The fatty acyl components of eight major peaks in *D. melanogaster* PE are shown in Table 1(A). The $[M-H]^-$ ion at *m/z* 742 of the PE logically includes a contribution from the $[M-15]^-$ ion of the PC (757 Da) in total lipids. In Fig. 3(B), a small ion at *m/z* 253 may come from that consisting of 16 : 1/18 : 1 by showing at *m/z* 253 and 281 (Fig. 5(B)). This indicates that there is very little contribution from PCs in the negative ion mode of *D. melanogaster* total lipid (Fig. 1(B)).

PC molecular species in *D. melanogaster*

In FAB-MS, PC molecules fragment to yield an ion at *m/z* 184 that represents the phosphocholine polar head group $[H_2PO_4—CH_2CH_2N^+(CH_3)_3]$. In Fig. 4(A) is shown a

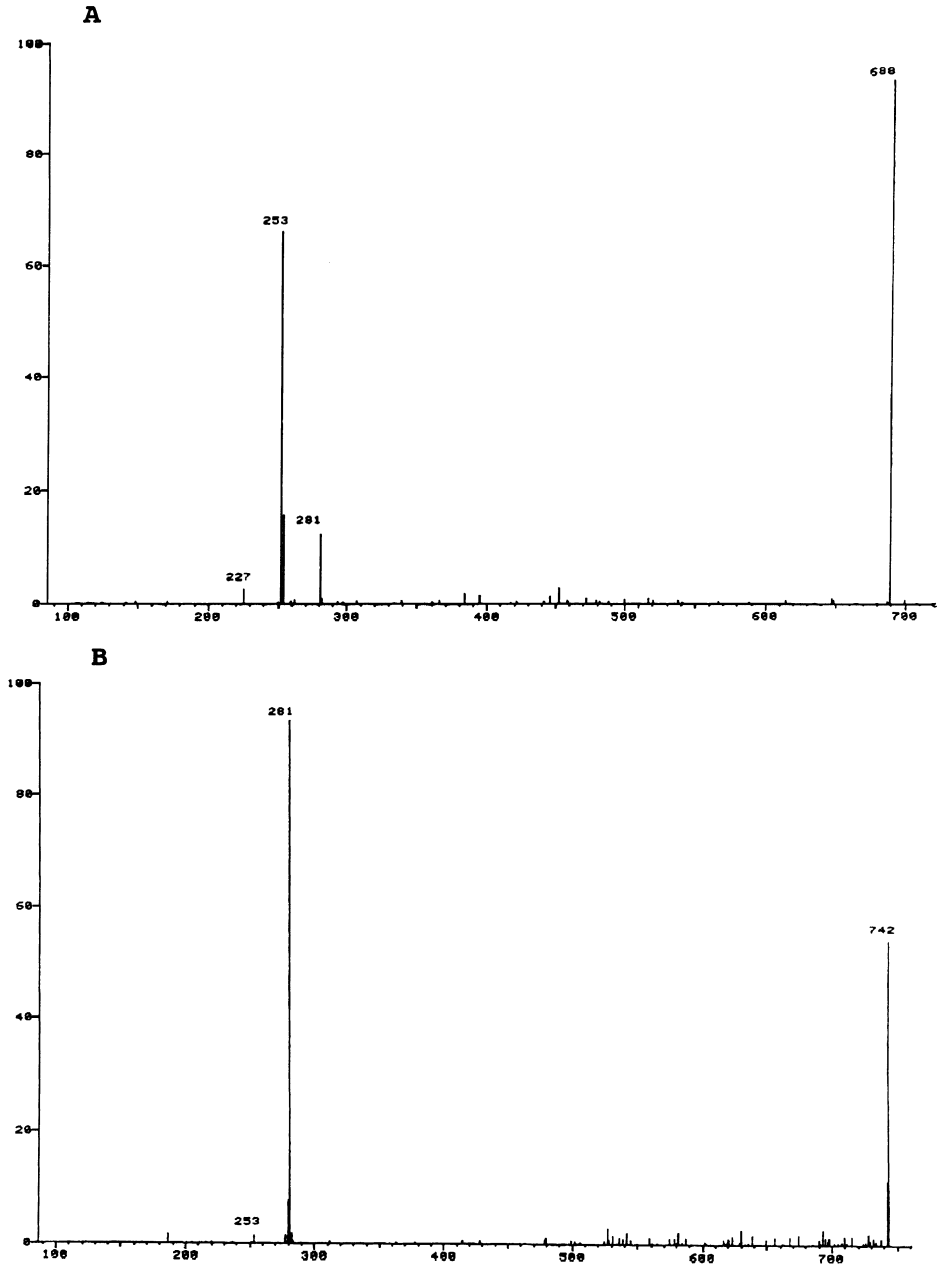

Figure 3. Product ion spectra of *D. melanogaster* PE: (A) at *m/z* 688 (16 : 0/16 : 1 = *m/z* 255 and 253, 14 : 0/18 : 1 = *m/z* 227 and 281) in purified PE fraction; (B) at *m/z* 742 (18 : 1/18 : 1 = *m/z* 281) in total lipids; (C) at *m/z* 740 (18 : 2/18 : 1 = *m/z* 279 and 281) in purified PE fraction from 5-day-old *Canton-S*; (D) at *m/z* 740 (18 : 1/18 : 2 = *m/z* 281 and 279) in purified PE fraction from 5-day-old Eth^{AR}*201*.

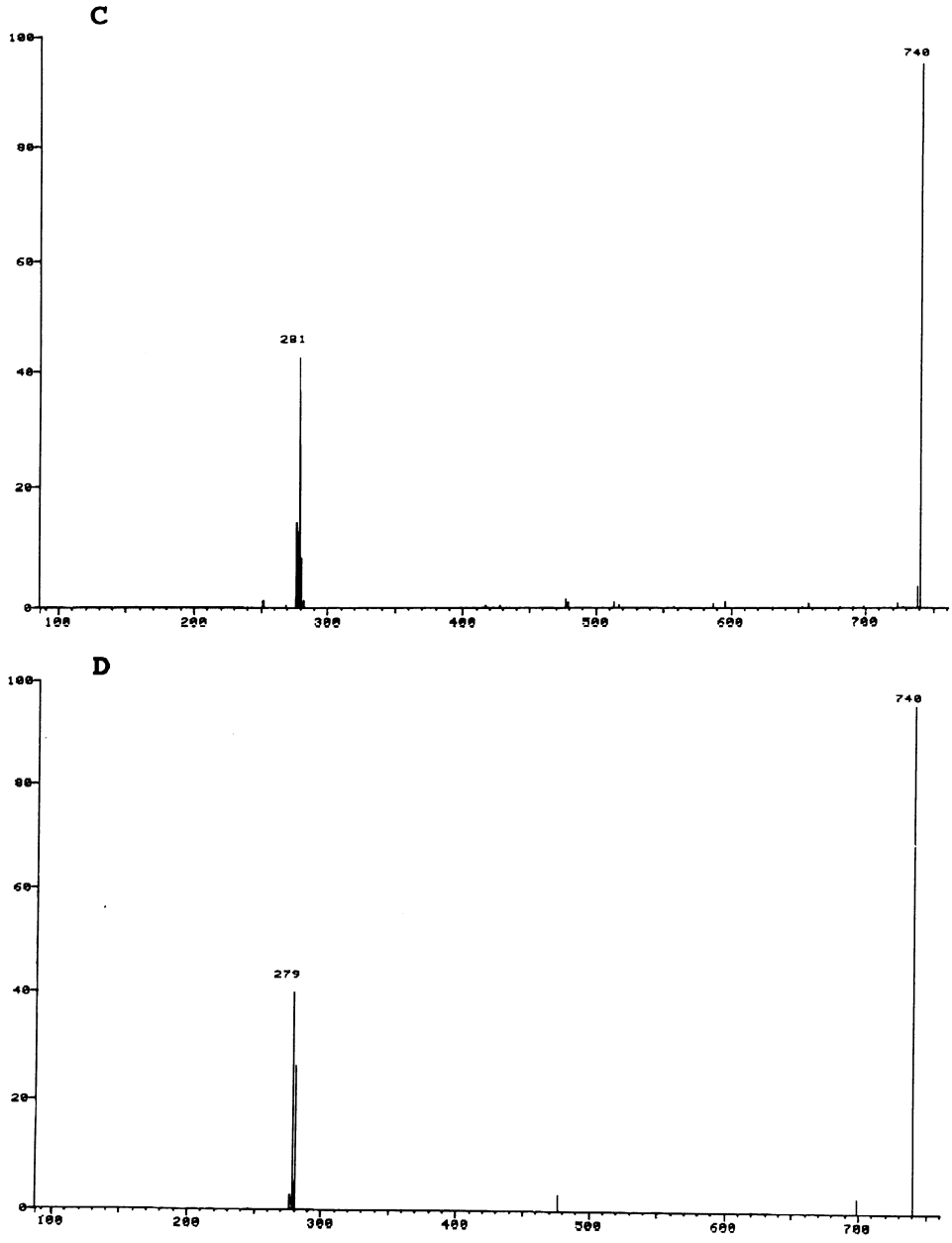

Figure 3. (*continued*).

Table 1. The major PE and PC molecular species in *D. melanogaster*

(A) PE				(B) PC				
$[M+H]^+$ *m/z*	*M*[a] (FAs[b])	*sn*-1/*sn*-2[c]	*sn*-1/*sn*-2[d]	$[M+H]^+$ *m/z*	*M*[a] (FAs[b])	$[M-15]^{-}$[e]	$[M-60]^{-}$[e]	$[M-86]^{-}$[e]
662	661(30 : 1)		—	704	703(30 : 1)	688	643	617
688	687(32 : 2)	16 : 1/16 : 1	—	730	729(32 : 2)	714	669	643
690	689(32 : 1)	16 : 0/16 : 1	14 : 0/18 : 1	732	731(32 : 1)	716	671	645
714	713(34 : 3)	16 : 1/18 : 1	—	756	755(34 : 3)	740	695	669
716	715(34 : 2)	16 : 1/18 : 1	16 : 0/18 : 2	758	757(34 : 2)	742	697	671
718	717(34 : 1)	16 : 0/18 : 1	—	760	759(34 : 1)	744	699	673
740	739(36 : 4)	18 : 2/18 : 2	—	782	781(36 : 4)	766	721	695
742	741(36 : 3)	18 : 2/18 : 1	—	784	783(36 : 3)	768	723	697
744	743(36 : 2)	18 : 1/18 : 1	—	786	785(36 : 2)	770	725	699

[a] *M* is molecular mass of PE and PC molecular species.
[b] FAs shows the total numbers of carbon atoms and double bonds of the two fatty acyls in PE and PC.
[c,d] The fatty acyl positions, *sn*-1/*sn*-2[c] and *sn*-1/*sn*-2[d] represent [c]major and [d]minor compositions in isobaric PE molecular species.
[e] The triplet ions $[M-15]^{-}$, $[M-60]^{-}$ and $[M-86]^{-}$ are from PC in negative FAB-MS (Figs 1(B) and 5(A)).

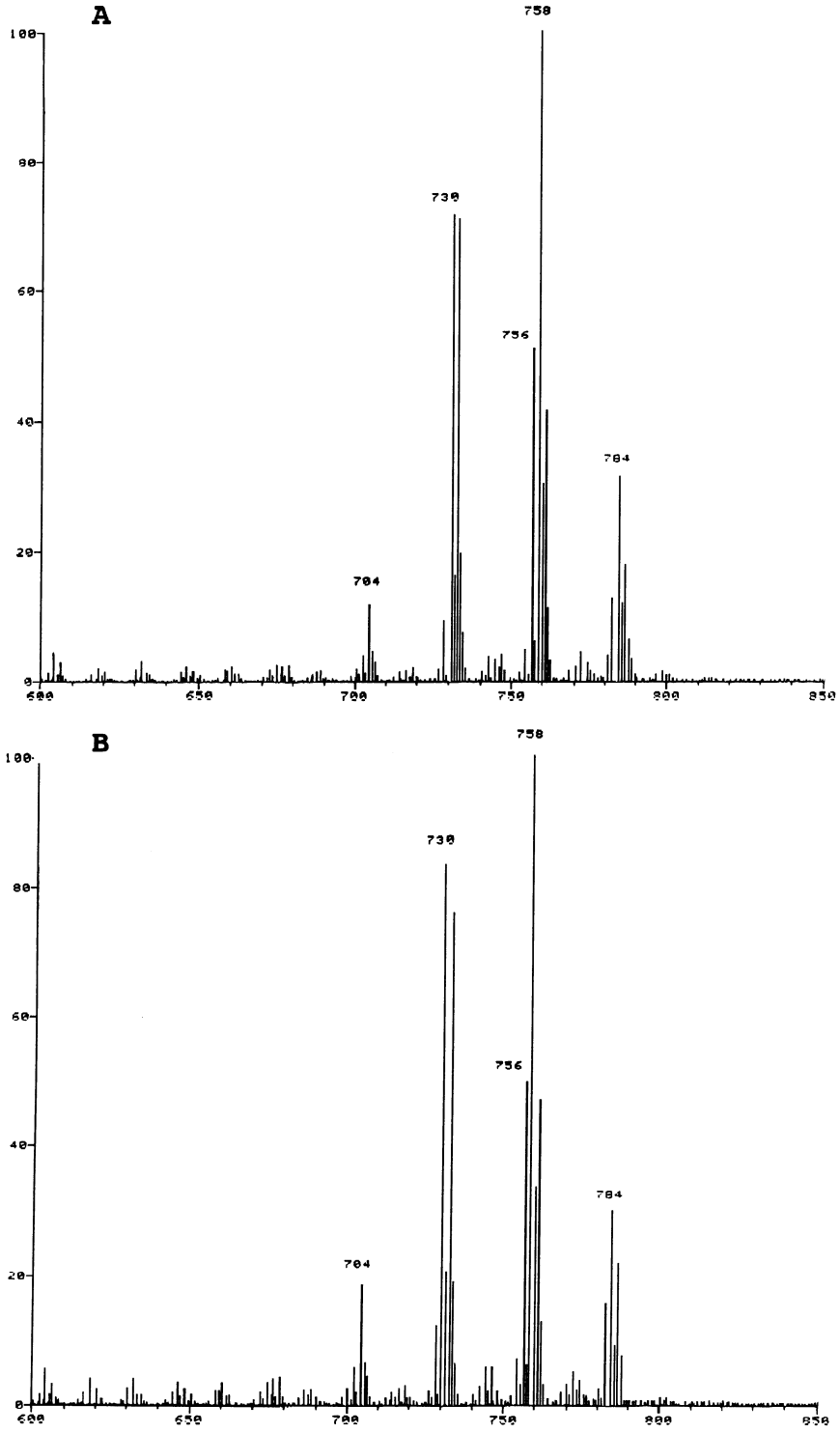

Figure 4. Precursor ion scan of *m/z* 184 for PC in *D. melanogaster*: (A) total lipids; (B) purified PC fraction from 5-day-old *Canton-S*. The percentage relative ion abundances between total lipids and PC fractions were not significantly different from each other by Student's *t*-test.

precursor ion scan of *m/z* 184 from the analysis of the total lipid extract; for comparison, Fig. 4(B) shown the FAB-MS analysis of the PC fraction isolated by TLC. The spectra were not significantly different from each other. Twelve 1,2-diacy1-PC in *D. melanogaster* were detected and their total carbon atoms and total double bonds in acyl groups were represented as $[M + H]^+$ ions at *m/z* 704 (30 : 1), 728 (32 : 3), 730 (32 : 2), 732 (32 : 1), 754 (34 : 4), 756 (34 : 3), 758 (34 : 2), 760 (34 : 1), 782 (36 : 4), 784 (36 : 3), 786 (36 : 2) and 788 (36 : 1) in 5-day-old flies. In our previous data concerning the PC molecular species of 1-day-old adult *D. melanogaster*, 30 : 1, 30 : 0, 32 : 2, 32 : 1, 32 : 0, 34 : 3, 34 : 2, 34 : 1, 36 : 5, 36 : 4 36 : 3 and 36 : 2 had been detected using GC and GC/MS.[11] In the negative ion mode (Fig. 5(A)), the PC species do not have $[M - H]^-$ ions but have the triplet ions $[M - 15, M - CH_3^+]^-$, $[M - 60, M - HN(CH_3)_3^+]^-$ and $[M - 86, M - HN(CH_3)_3^+ - C_2H_2]^-$.[20] The triplet ions are

listed in Table 1(B). The fatty acyl groups of a few molecular species in the PC fraction were determined by product ion scanning of the triplet ions. An $[M-15]^-$ ion at *m/z* 742 of a 757 Da (34 : 2) PC gave the two carboxylate fragments at *m/z* 253 and 281 (Fig. 5(B)). The carboxylate anion expelled from the 2-position has been shown to have a relative abundance about twice that of the acid arising from the 1-position of PCs by Jensen *et al*.[20] Therefore, 1-palmitoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16 : 1/18 : 1) is a major molecular species and a small amount of 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (16 : 0/18 : 2) is also included in the 757 Da PC.

PS and PI molecular species in *D. melanogaster*

In the PS fraction, the FAB tandem mass spectrum of the neutral loss scan that loses the phosphoserine head group [$H_2PO_4CH_2CH(NH_2)COOH$, *m/z* 185] from the $[M+H]^+$ ions is shown in Fig. 6(A). Nine $[M+H]^+$ ions at *m/z* 732 (32 : 2), 758 (34 : 3), 760 (34 : 2), 762 (34 : 1), 780 (36 : 6), 782 (36 : 5), 784 (36 : 4), 786 (36 : 3) and 788 (36 : 2) were detected in the neutral loss scan of *m/z* 185. Product ion scans of selected $[M-H]^-$ ions indicated the following compositions for *D. melanogaster* PS: *m/z* 758 (34 : 2, 16 : 1/18 : 1), 784 (36 : 3, 18 : 2/18 : 1) and 786 (36 : 2, 18 : 1/18 : 1, Fig. 6(B)). The loss of a dehydroserine

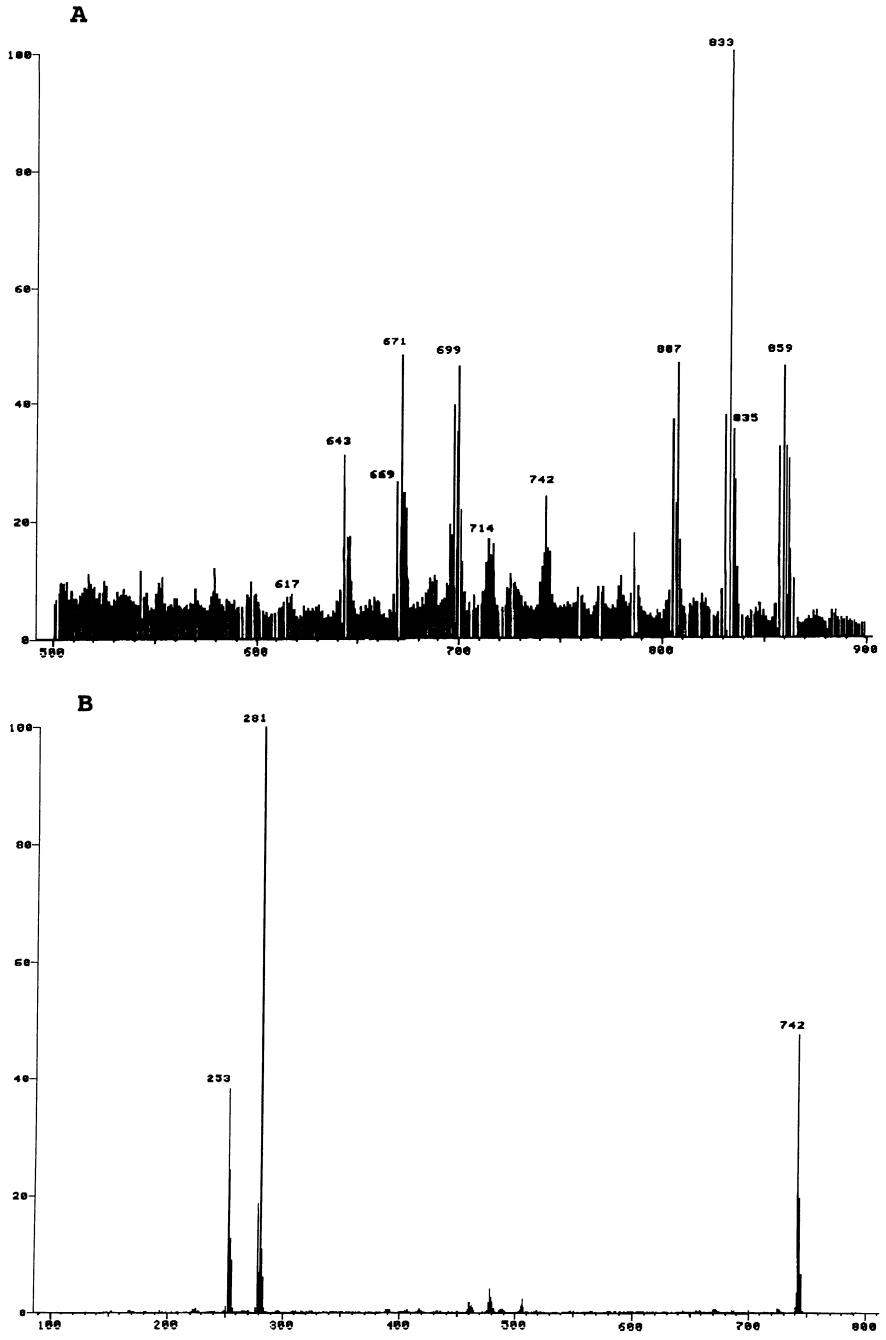

Figure 5. Negative mass spectra of PC and PI in *D. melanogaster*: (A) negative ion FAB-MS of the mixture of purified PC and PI fractions; (B) product ion scan of the $[M-15]^-$ ion at *m/z* 742 (16 : 1/18 : 1 = *m/z* 253 and 281, 16 : 0/18 : 2 = *m/z* 255 and 279) in purified PC fraction; (C) product ion scan of the $[M-H]^-$ ion at *m/z* 886 (20 : 4/18 : 0 = *m/z* 303 and 283) from pig liver PI (2 mg/ml^{-1} a phospholipid kit; Serdary Research, Laboratories, Ontario, Canada).

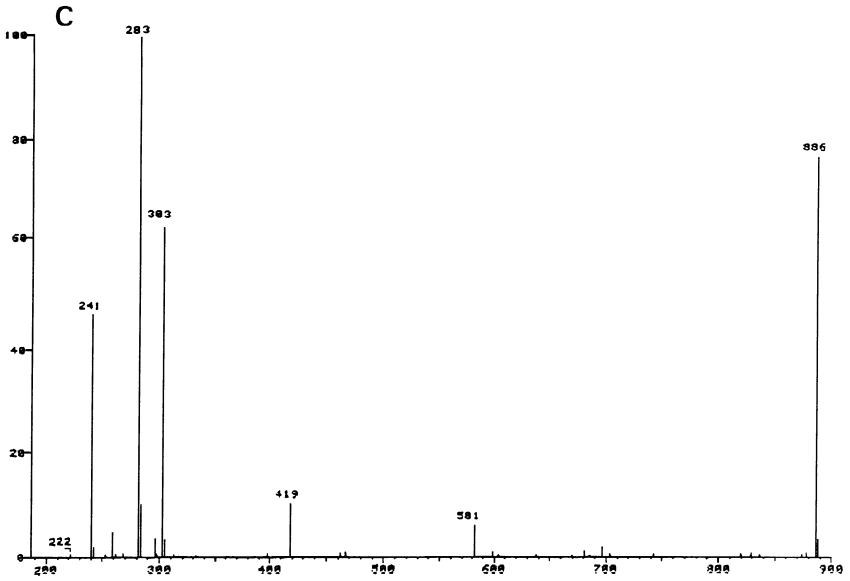

Figure 5. (*continued*).

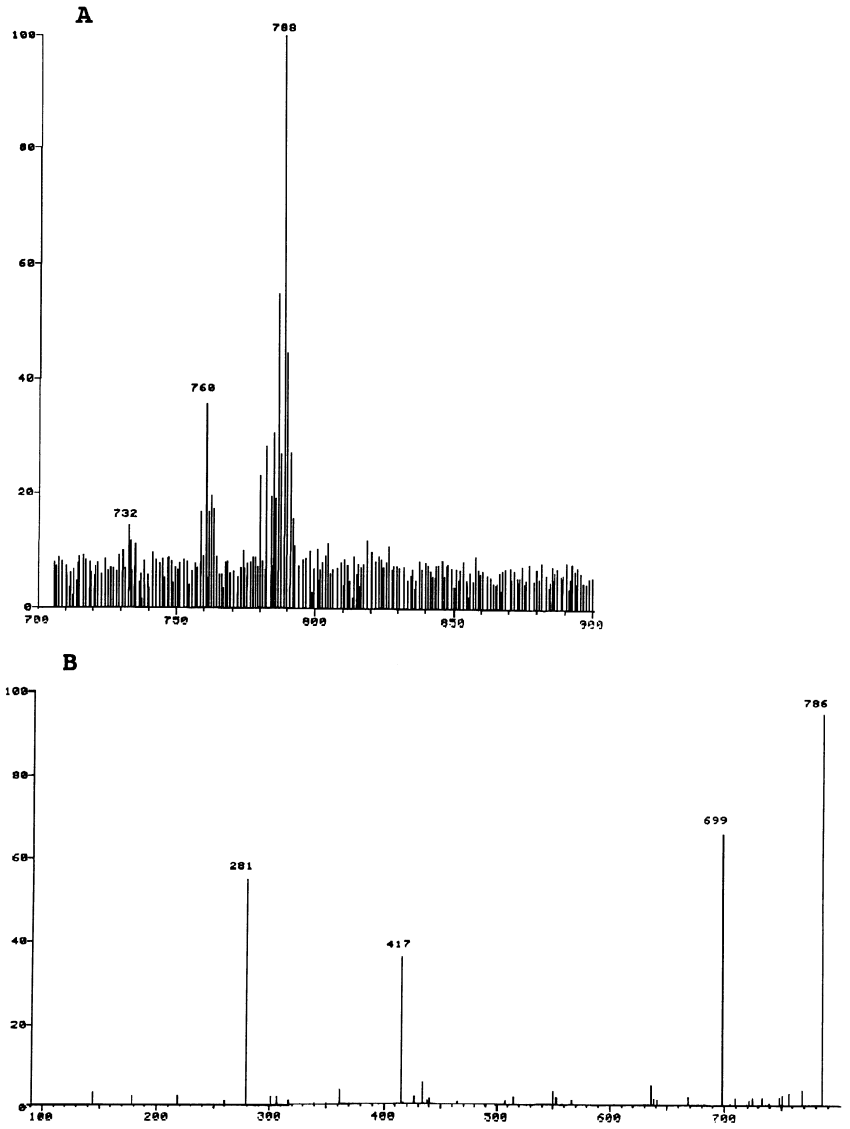

Figure 6. Mass spectra of PS in *D. melanogaster*: (A) neutral-loss scan of *m/z* 185 in purified PS fraction from 5-day-old *Canton-S*; (B) the product ion scan of the $[M - H]^-$ ion at *m/z* 786 (18 : 1/18 : 1 = *m/z* 281).

moiety from the precursor $[M-H]^-$ ion yields an $[M-H-87]^-$ ion at *m/z* 699 (Fig. 6(B)). The formation of this ion is an important marker for the presence of PSs.[20] In addition, the ion at *m/z* 417 presumably indicates the cleavage of the ester at the *sn*-1 in combination with loss of 74 Da ($NH_2CHCOOH$) from the polar head group. This ion is likely to provide further insight into the pattern of acyl group substitution in PS. In the positive and negative ion FAB-MS of glycerophospholipids (Figs 1(A) and 1(B)), the spectra naturally include a contribution from $[M+H]^+$ and $[M-H]^-$ of PSs, respectively.

In the negative FAB-MS of the PI fraction (Fig. 5(A), six $[M-H]^-$ ions at *m/z* 807 (32 : 1), 809 (32 : 0), 833 (34 : 2), 835 (34 : 1), 859 (36 : 3) and 861 (36 : 2) were observed. They are also detected in total lipids (Fig. 1(B)). Interpretation of the ions was made by comparison with FAB/mass and tandem mass spectra obtained from the analysis of commercially available pig liver PI (Fig. 5(C)). The product ion scan of the $[M-H]^-$ ion at *m/z* 886 showed ions at *m/z* 303 and 283, which correspond to 20 : 4/18 : 0 PI. The loss of one fatty acid (20 : 4) and the loss of 162 Da (inositol) from the precursor ion yield the ions at *m/z* 581 and 419, respectively. The ion at *m/z* 241 has been tentatively described as a dehydration form of *m/z* 259 (phosphoinositol-H).[24]

Effects of age and strain-dependent differences

The effects of age on wild-type *Canton-S* were examined and it was found that 713 (34 : 3), 739 (36 : 4) and 741 (36 : 3) Da PEs were significantly lower in relative abundances at 1 day old than at 5 and 20 days old, while 661 (30 : 1), 689 (32 : 1) and 717 (34 : 1) Da PEs were significantly higher in relative abundances at 1 day old than at 5 and 20 days old (Fig. 7(A)). However, these

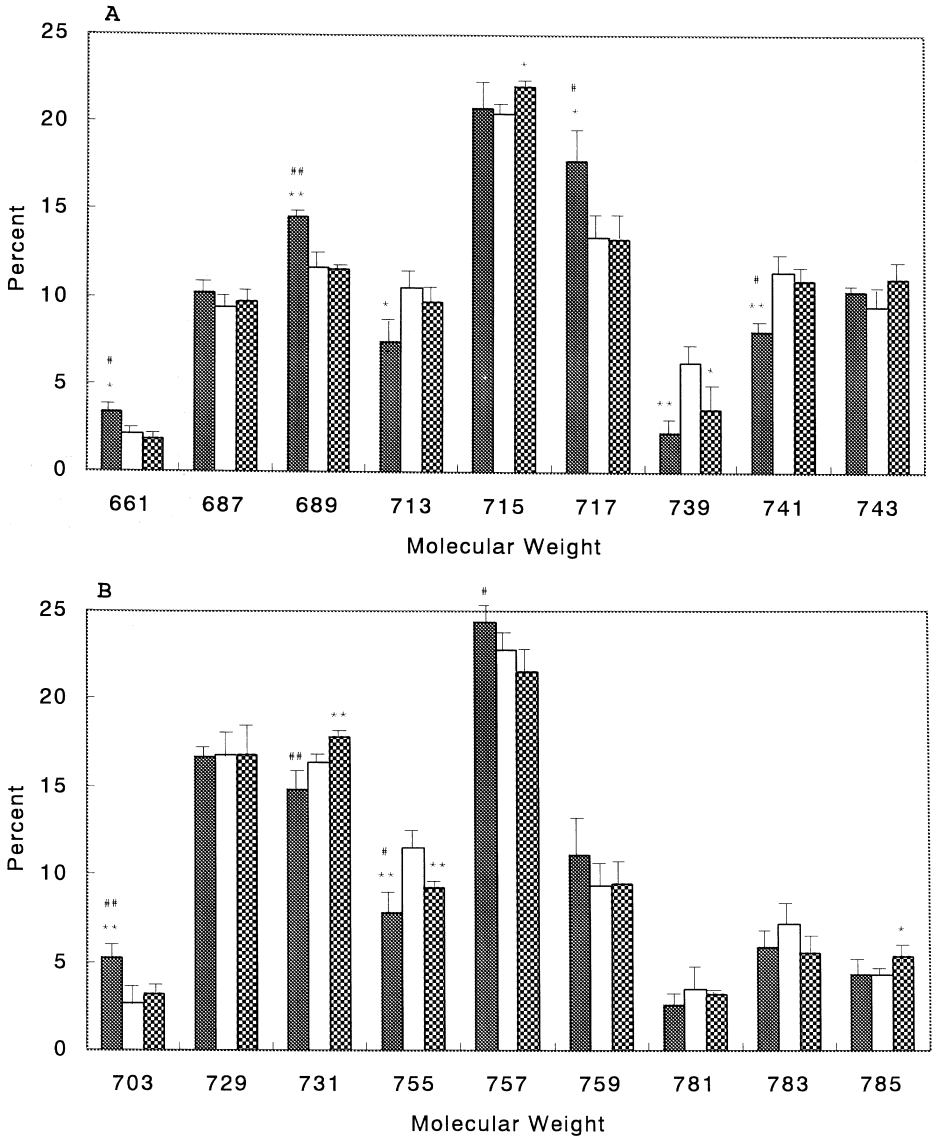

Figure 7. Effect of age on phospholipids in *D. melanogaster*: (A) PE molecular species using the neutral loss of *m/z* 141; (B) PC molecular species using the precursor ion scan of *m/z* 184. The vertical axis is the percentage relative ion abundance among 10 PEs and 12 PCs. The horizontal axis is the molecular masses (Da) of nine major PE and PC molecular species (Table 1). The average percentage relative ion abundances and standard deviations (SD) from four independent samples, *Canton-S* total lipids of 1 day (■), 5 days (□) and 20 days old (▩), were evaluated by Student's *t*-test. $^{*}P < 0.05$ and $^{**}P < 0.01$ represent the probabilities of differences from 5 days old and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ represent the differences from 20 days old.

relative abundances at 5 days old were similar to those for at 20-day-old flies except for 715 and 739 Da PEs. In young flies (1 day old), the PE molecular species with shorter carbon chain lengths and fewer double bonds in the acyl groups show higher ion abundances, but longer carbon chain lengths and highly unsaturated fatty acyl groups show lower ion abundances than in older flies. Age effects on PC molecular species were also found with some exceptions (Fig. 7(B)). The 20-day-old flies showed higher ion abundance at 731 Da (32 : 1) and lower ion abundance at 757 Da (34 : 2) than younger flies. These age effects may reflect the enzyme activities in desaturase as well as fatty acid and phospholipid metabolism pathways. We found the fact that the activities of diacylglycerol–cholinephosphotransferase and diacylglycerol–ethanolaminephosphotransferase were three times higher in 1-day-old flies than in 5-day-old flies but there was no difference between the ether-resistant and -sensitive strains.[23]

In two wild-types, *Canton-S* and *Oregon-R*, and one mutant of the ether-resistant strain, Eth^{AR}201, the total lipids of 5-day-old flies were analyzed to determine PE and PC molecular species by using FAB-MS/MS. When the three strains were compared, strain-dependent differences in the PE molecular species were not found between the two wild-type strains. However, a few molecular species in Eth^{AR}201 showed a difference in relative abundance from the wild-types (Fig. 8(A)). Only a 703 Da PC was higher in relative abundance in *Oregon-R* than in other strains (Fig. 8(B)). Variations among the *D. melanogaster* strains in PE and PC are smaller than the effects of age.

One difference between the wild-types and Eth^{AR}201 lies in a pair of isobaric molecular PE of 741 Da. The two fatty acyls were speculated to be 18 : 2 at *sn*-1 and 18 : 1 at *sn*-2 by showing ions at *m*/*z* 279 and 281, respectively, in the wild-types (Fig. 3(C)), but the acyl groups were 18 : 1 at *sn*-1 and 18 : 2 at *sn*-2, because the ion at *m*/*z*

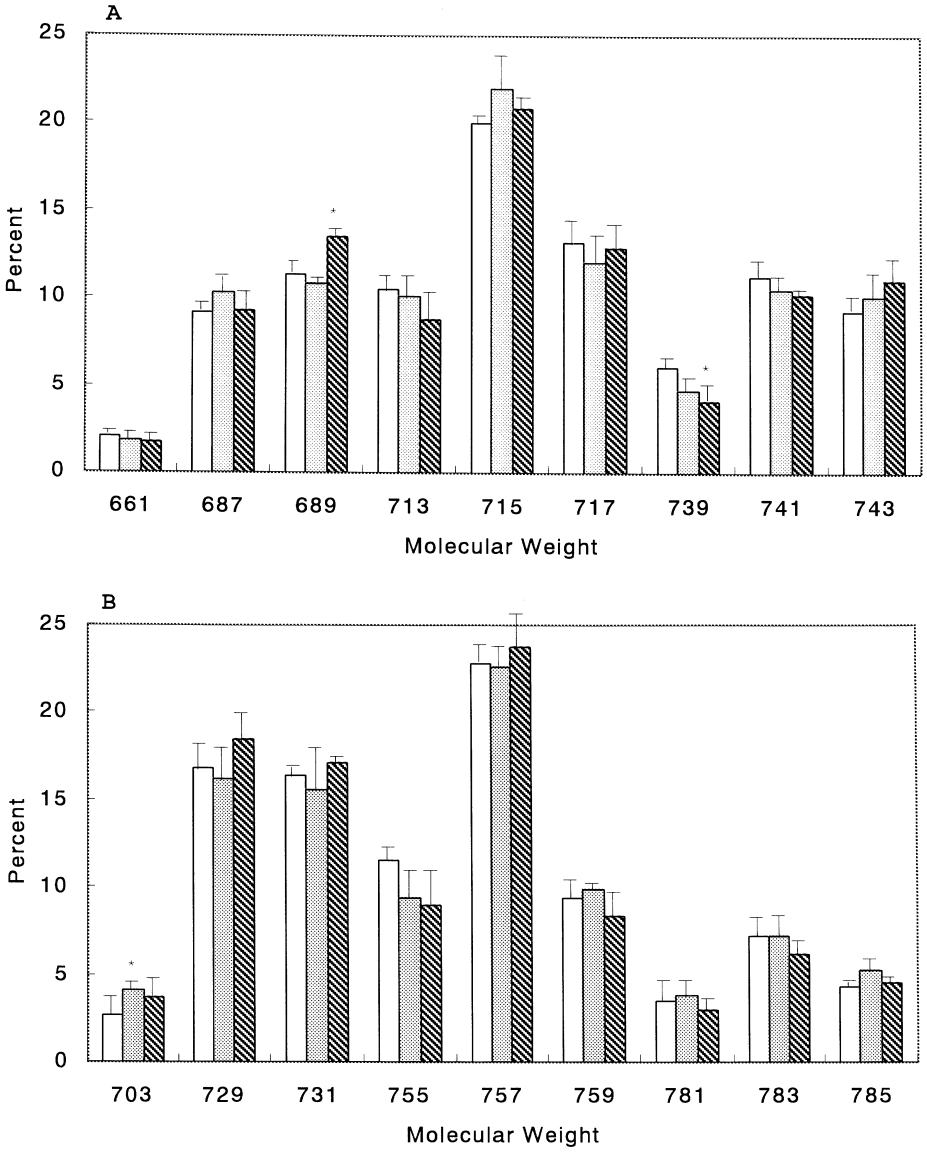

Figure 8. Strain-dependent differences in phospholipids of *D. melanogaster*: (A) PE molecular species; (B) PC molecular species. Other comments as in Fig. 7. The average percentage of relative ion abundances and SD from four independent samples, total lipids of 5-day-old flies among *Canton-S* (□), *Oregon-R* (▨) and Eth^{AR}201 (▩) were evaluated by Student's *t*-test. $^{*}P < 0.05$ represents the probability of differences from *Canton-S*.

279 showed a higher abundance than that at *m*/*z* 281 in *Eth*^{AR}201 (Fig. 3(D)). The ether-resistant strain may be a mutant of a PE molecular species. Further experiments on FAB-MS/MS combined with genetics are needed to determine whether this difference is genetically related to the phenotype, which is ether resistant.

D. melanogaster has been used as a material in molecular genetics, but few studies on phospholipids have been reported.[11–15] For the screening of mutants in phospholipid molecular species, the total lipids from only one fly (1 mg) is enough for several FAB-MS and/or FAB-MS/MS analyses of PE and PC. However, PS and PI, which are present in small amounts in *D. melanogaster*, need lipid class separation by TLC prior to FAB-MS. The product ion scans of phospholipids also need TLC separation except major PE molecular species, for which the total lipids may be used. We recommend the use of at least, 10 flies (10 mg) as a sample for mutant screening.

CONCLUSIONS

FAB-MS in combination with neutral loss scanning of *m*/*z* 141 for PE and *m*/*z* 185 for PS and precursor ion scanning of *m*/*z* 184 for PC was applied to the identification of *D. melanogaster* glycerophospholipids. Information concerning the polar head group and the two fatty acid substituents at *sn*-1 and *sn*-2 was obtained for eight PEs, one PC and three PS molecular species from about 50 phospholipid molecular species. The predominant ion species were PC and PE when positive and negative ion detection were used, respectively. Analyses of unfractionated phospholipids provided as much information as TLC-purified sample analyses by the use of the appropriate MS/MS scan modes.

The FAB-MS/MS techniques were applied to the analysis of the effects of age- and strain-dependent differences in PE and PC molecular species. We found age effects in some PE and PC molecular species; those with shorter carbon chain lengths and fewer points of unsaturation in fatty acyl groups (30 : 1, 32 : 1 and 34 : 1 PEs and 30 : 1 PC) were more abundant, while those with longer carbon chain lengths and more unsaturated fatty acid groups (34 : 3, 36 : 4 and 36 : 3 PEs and 34 : 3 PC) were less abundant in young (1-day-old) flies than in older (5- and 20-day-old) flies.

We found a difference in the 741 Da PE between the ether resistant mutant *Eth*^{AR}201 and the wild types; in the wild-types, 18 : 2/18 : 1 was prominent, but 18 : 1/18 : 2 was found, instead, in *Eth*^{AR}201.

From these results the FAB-MS/MS method not only facilitates the analysis of the phospholipid molecular species but also the screening of the phospholipid molecular species mutations. However, it is important that flies of the same age are used as the sample for the screening.

REFERENCES

1. N. J. Jensen and M. L. Gross, *Mass Spectrom. Rev.* **7**, 41 (1988).
2. T. Matubara and A. Hayashi, *Prog. Lipid Res.* **30**, 301 (1991).
3. R. N. Pickard, J. C. Ludwig and L. M. McManus, in *Inflammation*, edited by J. I. Gallin, I. M. Goldstein and R. Snyderman, 139. Raven Press, New York (1988).
4. M. L. Blank, F. Snyder, L. W. Byers, B. Brooks and E. E. Murirhead, *Biochem. Biophys. Res. Commun.* **90**, 1194 (1979).
5. A. B. Jefferson and V. S. Bansal, *Cell* **63**, 459 (1990).
6. J. T. O'Flaherty, *Lab. Invest.* **47**, 314 (1982).
7. R. F. Irvine, *Biochem. J.* **204**, 3 (1982).
8. M. J. Cole and C. G. Enke, *Anal. Chem.* **63**, 1032 (1991).
9. K. A. Kayganich and R. C. Murphy, *Anal. Chem.* **64**, 2965 (1992).
10. K. A. Kayganich-Harrison and R. C. Murphy, *Anal. Biochem.* **221**, 16 (1992).
11. S. Gamo, E. Nakashima-Tanaka and M. Ogaki, *Life Sci.* **30**, 401 (1981).
12. T. Yoshioka, H. Inoue and Y. Hotta, *Biochem. Biophys. Res. Commun.* **111**, 567 (1983).
13. W. S. Stark, T. N. Lin, D. Brackhahm, J. S. Christianson and G. Y. Sun, *Lipids* **28**, 23 (1993).
14. H. E. Jones, J. L. Harwood, I. D. Bowen and G. Griffiths, *Lipids* **27**, 984 (1992).
15. P. Pavlidis, M. Ramaswami and M. Tanouye, *Cell* **79**, 23 (1994).
16. D. L. Lindsley and G. G. Zimm, *the Genome of Drosophila*. Academic Press, New York (1992); E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
17. S. Gamo, F. Taniguchi, K. Morioka, Y. Tanaka, M. Michinomae and Y. Inoue, *Prog. Anesth. Mech.* **2**, 11 (1994).
18. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
19. J. D. Dittmer and R. J. Lester, *J. Am. Oil Chem. Soc.* **48**, 752 (1964).
20. N. J. Jensen, K. B. Tomer and M. L. Gross, *Lipids* **21**, 580 (1986).
21. Z. H. Huang, D. A. Gage and C. C. Sweely, *J. Am. Soc. Mass Spectrom.* **3**, 71 (1991).
22. F. H. Chilton, III, and R. C. Murphy, *Biomed. Environ. Mass Spectrom.* **13**, 71 (1988).
23. S. Gamo, H. Iwanaga and E. Nakashima-Tanaka, *Genetics* **110**, s91 (1985).
24. A. A. Karlsson, P. Michelsen, A. Larsen and G. Odham, *Rapid Commun. Mass Spectrom.* **10**, 775 (1996).