Mass spectrometry of the phosphatidyl amino alcohols: detection of molecular species and use of low voltage spectra and metastable scanning in the elucidation of structure

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Abstract The basic fragmentation mechanisms occurring in the mass spectra of the phosphatidylcholines have been described previously, and evidence was adduced to show that many of the more abundant ions are related by electron impact-induced processes. A molecular ion was demonstrated for dioleoyl glycerylphosphorylcholine by accurate mass measurement and by metastable scanning.

In the present paper, results are reported which further extend the previous work by including a more detailed investigation of "nonapparent" fragment ions for a series of phosphatidylcholines with different acyl side chains and also for a series of phosphatidyl amino alcohols of fixed acyl composition. The results demonstrate the effect of the choline quaternary nitrogen on the stability of the molecular ion, and estimates for the appropriate rate constants are given. Nitrogen-containing fragments have been demonstrated by recording spectra at low electron voltages.

The work has also been extended to include natural phosphatidylcholine preparations of mixed acyl composition, and the possibility of detecting particular molecular species has been established. Quantitative estimates may be made using suitable synthetic phosphatidylcholines, and results are presented to show the variation of the molar correction factor with acyl chain length.

Supplementary key words "nonapparent" fragment ions · quaternary nitrogen · amino alcohols · low electron voltage · natural mixtures

METASTABLE SCANNING in the first field-free region may be used to obtain a considerable amount of information concerning very short-lived ions that fragment before reaching the second field-free region or the collector. Shadoff (1) demonstrated that it was possible to detect "nonexistent" ("nonapparent" would be more precise) molecular ions by metastable scanning in the 1FFR. It has been estimated that lifetimes of about 3 μsec are necessary for the decomposition to be observed in the 1FFR, and about 10 μsec for the decomposition to be observed in the 2FFR (2, 3), with an ion of m/e 101 at accelerating potentials of 5 kv and 8 kv, respectively. For a metastable transition to be recorded, the ion must at least leave the source. Ions with lifetimes of 1 μsec or less will fail to leave the source, and only the fragmentation product will be recorded at the collector.

This technique has been used extensively in the present work to provide information about the molecular ion and other ions in the spectra of the phosphatidylcholines and other phosphatidyl amino alcohols. These ions are of such low intensity as not to be recorded at the collector unless the instrument is operated in the defocused mode (4–6). For all the phosphatidylcholines, the ion resulting from loss of the phosphorylcholine moiety (structures 1, I, and A in Ref. 7) is found to be related to the molecular ion (M^+) by an electron im-

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; 1FFR and 2FFR, first and second field-free regions; diacyl GPX, 1,2-diacyl glycerylphosphoryl-X, where X may be choline (C), *N*,*N*-dimethylethanolamine (DME), *N*-methylethanolamine (ME), or ethanolamine (E); PC, phosphatidylcholine; PDME, phosphatidyl *N*,*N*-dimethylethanolamine; PME, phosphatidyl *N*-methylethanolamine; PE, phosphatidylethanolamine; CBZ, carbobenzoxy; TMS, trimethylsilyl; EPL, essential phospholipid.

pact-induced process, as well as to ions resulting from the loss of the trimethyl nitrogen group from the molecular ion and from cleavage of the oxygen–phosphorus bond nearest to the glycerol backbone.

An examination of the metastable spectra for various homologs of the phosphatidylcholines with substitution at the nitrogen atom has demonstrated that the molecular ion was not detectable, indicating that the ion lifetime was insufficient for the ion to leave the source and that the quaternary nitrogen atom in the phosphatidylcholines must exert a considerable influence on the stability of the molecular ion. The ion resulting from O–P cleavage was present, however, for all the compounds studied.

The recording of mass spectra at low electron voltage results in an accentuation of ions arising from rearrangement reactions (8) and also of those ions containing a charge center of low ionization potential (9). In the spectra of the phosphatidylcholines, many of the ions (at high *m/e*) are resonance-stabilized and are therefore particularly apparent at low electron voltage. Fragment ions at the low-mass end of the spectrum contain nitrogen in addition to carbon, hydrogen, and oxygen. Reduction of the electron voltage enhances those ions containing nitrogen (10) because the ionization potential for a nitrogen atom is less than that for an oxygen atom (ionization potential for $C_2H_5NH_2$ is 9.4–9.6 ev; for C_2H_5OH it is 10.7 ev [11]); at the same time it drastically reduces the contribution from hydrocarbon fragments (e.g., *m/e* 55, represented as cyclobutene $\square^+$: 11.9[9] ev [10]).

The use of low voltage spectra enables one to identify nitrogen-containing fragments from the amino alcohol moiety in the intact phospholipid. Duncan, Lennarz, and Fenselau (12) have approached this problem by using the trimethylsilyl derivatives of the glycerophosphate ester after deacylation of the glycerophospholipid with methanolic sodium hydroxide.

It has been shown by Perkins and Johnston (13) and by Horning, Casparrini, and Horning (14) that when phospholipids are subjected to thermal cleavage and gas-phase analytical procedures (GLC), only derivatives of the diglyceride moiety are observed. These authors have demonstrated the identity of the products by the technique of mass spectrometry. Considerable positional scrambling was observed between the 1 and 2 positions, depending for its extent on the conditions used for thermal cleavage.

Results reported in this paper show that it is possible to demonstrate the presence of fragments related to the various molecular species in natural phosphatidylcholine mixtures by mass spectrometric analysis of the purified phospholipid class without further chemical manipulation.

MATERIALS AND METHODS

Mass spectra were recorded on an Associated Electrical Industries MS902 double-focusing mass spectrometer, which was operated under the same conditions as those reported previously (7). Metastable spectra were obtained with the instrument in the defocused mode (10), at an accelerating voltage of 4 kv. Samples dissolved in chloroform were deposited on the probe.

Synthetic 1,2-dipalmitoyl-L- and -DL-glycerylphosphorylethanolamine and 1,2-dipalmitoyl-DL-glycerylphosphorylcholine were obtained from Sigma Chemical Co. Ltd., London. 1,2-Dipalmitoyl-L-glycerylphosphoryl-*N*,*N*-dimethylethanolamine was obtained from Koch-Light Laboratories, Colnbrook, Bucks., England. These preparations were purified by silicic acid column chromatography before use.

1,2-Dipalmitoyl-L-glycerylphosphorylcholine was synthesized in this laboratory by the method of Baer and Buchnea (15). 1,2-Dipalmitoyl glycerylphosphate (phosphatidic acid) was prepared from dipalmitoyl GPC using the phospholipase D (EC 3.1.4.4) from savoy cabbage supernate according to the method of Davidson and Long (16). The crude phosphatidic acid was purified by a method similar to that used by Kornberg and McConnell (17), except that certain differences were found to be necessary. The following modifications were made: (*a*) the barium–phosphatidic acid complex was dissociated with HCl rather than with H_2SO_4, as the precipitate of barium sulfate was found to be difficult to manipulate; (*b*) extractions were carried out using the chloroform–methanol procedure of Folch, Lees, and Sloane Stanley (18) rather than with chloroform alone; (*c*) divalent cationic contaminants were removed by washing the chloroform phase with a solution containing 145 mM sodium chloride and 1 mM disodium ethylenediaminetetraacetic acid before preparative column chromatography on silicic acid CC4 (Mallinckrodt) with 4% methanol in chloroform as the solvent. The resulting phosphatidic acid gave a single spot on a heavily loaded silica gel H plate in both chloroform–methanol–water 65:25:4 (19) and chloroform–methanol–7 M ammonia 690:270:45 (20).

1,2-Dipalmitoyl-L-glycerylphosphoryl-*N*-methylethanolamine was prepared from dipalmitoyl glycerylphosphate by condensation with *N*-carbobenzoxy-*N*-methylethanolamine in chloroform–pyridine solution containing 2,4,6-triisopropylbenzenesulfonyl chloride as the condensing agent (21, 22). The *N*-carbobenzoxy-*N*-methylethanolamine was prepared by the method of Baer and Pavanaram (23). The carbobenzoxy group was removed from the condensation product by catalytic reduction with H_2 over 5% palladium on activated charcoal (24) in glacial acetic acid, at about 20 cm Hg

above atmospheric pressure. The glacial acetic acid was removed by evaporation under reduced pressure, and the dipalmitoyl glycerylphosphoryl-*N*-methylethanolamine was purified by column chromatography on silicic acid. Analysis of the final product by TLC revealed a single spot.

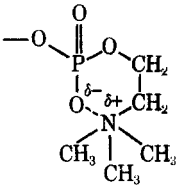
Phosphatidylcholine fractions A–D were prepared by fractionation of natural mixtures according to molecular species on columns of silicic acid (25). Fractions A and B were obtained from "essential phospholipid" (EPL), which is particularly rich in linoleic acid and is prepared from soybeans (the sample of EPL was generously donated by Dr. H. Eikermann, Nattermann and Cie, Cologne, West Germany). Fractions C and D were prepared from egg yolk phosphatidylcholine obtained from chickens fed a diet containing large amounts of polyunsaturated fatty acids (25).

Chromatographically homogeneous ox brain sphingomyelin was prepared and donated by N. G. A. Miller, Institute of Animal Physiology, Babraham, Cambridge, England. All of the phospholipids prepared by the methods outlined above gave a single spot on heavily loaded TLC plates when run in two solvents. Fatty acid methyl esters were prepared for GLC by transesterification of the phospholipids in methanolic sodium hydroxide (26).

RESULTS AND DISCUSSION

The metastable transitions observed in the 1FFR for various 1,2-diacyl glycerylphosphorylcholines are shown in Table 1. "P" indicates the fragment remaining after loss of the phosphoryl base moiety from the intact molecule; it is probably a resonance-stabilized cyclic structure (10). The ion most likely to correspond to the observed transition is shown in parentheses. The resolution is insufficient to distinguish between $(P + 17)^+$ and $(P + 16)^+$ or $(P + 18)^+$, or between $(M - 58)^+$ and $(M - 59)^+$. Similar data for a number of 1,2-diacyl glycerylphosphoryl-(*N* substituted)-ethanolamines are shown in Table 2. Relative intensities for the metastable ions are given in the form $(m^*)/(A^+)$, where (m^*) is the metastable ion intensity and (A^+) is the daughter ion intensity.

The presence of a molecular ion was inferred for all the phosphatidylcholines from the observation of an appropriate metastable ion. The intensity of this ion was generally low, about 1×10^{-3}. No such metastable ions related to the molecular ion were observed for the phosphatidyl-(*N* substituted)-ethanolamines. One possible explanation might be that the phosphatidylcholine is internally stabilized, with complete or partial charge neutralization:



The phosphatidyl-(*N* substituted)-ethanolamines do not produce molecular ions that are stable enough to leave the source before fragmentation occurs, and one must conclude that the quaternary nitrogen of the choline group confers a degree of stability upon the phosphatidylcholine molecular ion. Compare the effect on ion stability of substituting the *N*-carbobenzoxy group, as shown by the results in Table 2.

TABLE 1. Metastable transitions observed in the 1FFR for various 1,2-diacyl glycerylphosphorylcholines

Diacyl GPC	P^{+a}	$(P + 17)^{+b}$	$(M - 59)^{+b}$	M^+
Distearoyl	606.6	623.2 (623.6)	730.7 (730.6)	787.4 (789.6)
		32×10^{-3}	2×10^{-3}	0.2×10^{-3}
1-Stearoyl	604.5	620.7 (621.5)	726.8 (728.6)	783.5 (787.6)
2-Oleoyl		4×10^{-3}	0.5×10^{-3}	0.1×10^{-3}
1-Oleoyl	604.5	622.1 (621.5)	726.0 (728.6)	787.6 (787.6)
2-Stearoyl		11.2×10^{-3}	2.5×10^{-3}	1.2×10^{-3}
Dioleoyl	603.5	621.6 (620.5)	728.9 (726.6)	787.1 (785.6)
		$<0.1 \times 10^{-3}$	3×10^{-3}	0.1×10^{-3}
Dipalmitoyl	551.5	570.0 (568.5)	676.1 (674.5)	734.6 (733.6)
		8.6×10^{-3}	1.7×10^{-3}	0.6×10^{-3}
Dimyristoyl	494.4	512.2 (511.4)	618.1 (618.4)	676.3 (677.5)
		3×10^{-3}	1×10^{-3}	1×10^{-3}

The experimentally determined value for the mass of the parent ion is followed by the calculated value (in parentheses) most likely to correspond with the experimental value. Relative intensities are given in the form $(m^{*+})/(A^+)$, where (A^+) is the daughter ion intensity.

[a] Determined by accurate mass measurement.

[b] Resolution in the defocused mode is insufficient to distinguish between $(P + 16)$, $(P + 17)$, or $(P + 18)$, and $(M - 58)$ or $(M - 59)$.

TABLE 2. Metastable transitions observed in the 1FFR for various phosphatidyl (*N* substituted) bases

Base[a]	P^{+b}	$(P + 17)^{+c}$	$(M - 107)^+$	$(M - 91)^+$	$(M - 77)^+$	$(M - 15)^+$
N,N-Dimethyl GPE	550.5	568.6 (567.5) 10×10^{-3}				
N-Methyl GPE	550.5	566.3 (567.5) 5.5×10^{-3}				
N,N-CBZ-methyl GPE	550.5	566.5 (567.5) 10×10^{-3}	736.6 (732.6) 0.2×10^{-3}	747.6 (748.6) 0.2×10^{-3}	762.4 (762.6) 0.3×10^{-3}	824.7 (824.6) 1×10^{-3}
GPE	550.5	566.7 (567.5) 8×10^{-3}				
Phosphatidic acid	550.5	566.5 (567.5) 2×10^{-3}				
Dimyristoyl GPE	494.4	510.5 (511.4) 3×10^{-3}				

The experimentally determined value for the mass of the parent ion is followed by the calculated value (in parentheses) most likely to correspond with the experimental value. Relative intensities are given in the form $(m^{*+})/(A^+)$, where (A^+) is the daughter ion intensity.

[a] 1,2-Dipalmitoyl glycerylphosphoryl base, except for dimyristoyl GPE.

[b] Determined by accurate mass measurement.

[c] Resolution is insufficient to distinguish (P + 16), (P + 17), or (P + 18).

For an ion to be detected at all it must at least leave the source (1). In the present case this would imply an ion lifetime in excess of 0.5 μsec. An ion lifetime of 1–5 μsec will result in decomposition of the ion in the 1FFR with a metastable transition that may be detected by operating the spectrometer in the defocused mode (4–6), whereas a mean lifetime of 15–35 μsec results in the decomposition taking place in the 2FFR with a metastable ion that appears at an apparent mass of m_2^2/m_1 (27). Metastable transitions in the 2FFR were observed at an accelerating potential of 8 kv with the instrument used in this work, whereas those occurring in the 1FFR were observed with the spectrometer operating in the defocused mode at a reduced accelerating voltage of 4 kv.

The intensity of any ion recorded at the collector will depend on the equilibrium attained between formation and decomposition reactions and the time taken to travel the analyzer flight path. A detailed calculation is possible only for very simple decompositions with a limited and known number of competing pathways. For polyatomic molecules such a calculation may represent a gross and perhaps unwarranted simplification. It may be used, however, to demonstrate the effect of the rate constant on the abundance of metastable ions in the two field-free regions (3, 28). Any calculations based on such a treatment will yield an *apparent specific rate constant*, which will represent the mean, $\bar{k}$, of a continuum of rate constants (3, 29).

The intensity ratios for the metastable ions in the 1FFR obtained with the phosphatidylcholines are shown in Table 1. Assuming a unimolecular decomposition (3, 28, 30), rate constants of 1.7–2.3 $\times 10^7$ sec^{-1} for $M^+ \rightarrow P^+$ and 0.9–1.4 $\times 10^7$ sec^{-1} for $(P + 17)^+ \rightarrow P^+$ are appropriate.

Dipalmitoyl GPC exhibits many breakdown products associated with P^+ (*m/e* 551), as shown by the presence of metastable ions in the 2FFR (7). Dioleoyl and 1-stearoyl-2-oleoyl GPC show metastable transitions in the 2FFR also, but these are less in number. Values of 3.4–4.7 $\times 10^5$ sec^{-1} for the *apparent specific rate constants* are obtained for these decompositions, showing that the molecular ion decomposes at a rate approximately two

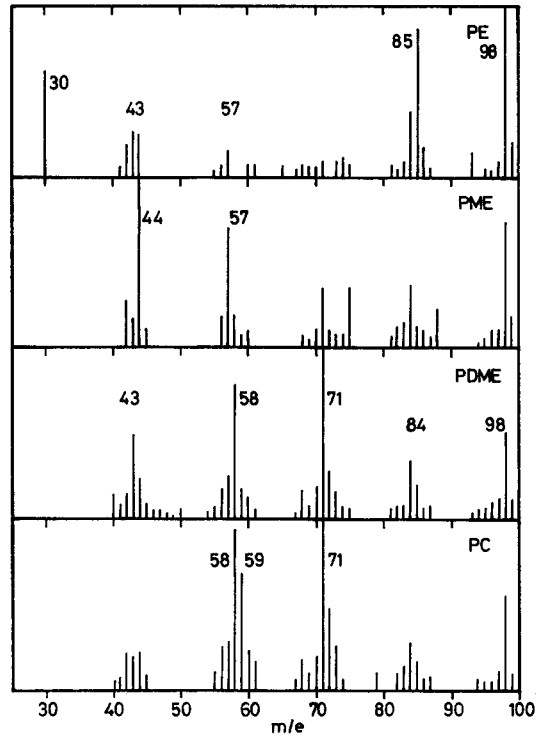


FIG. 1. Mass spectra for various phosphatidyl bases, recorded at 14 ev, showing *m/e* values between 25 and 100. Intensities are relative to the largest peak in this region. Peaks smaller than 1% are not shown. PE, phosphatidylethanolamine; PME, phosphatidyl-*N*-methylethanolamine; PDME, phosphatidyl-*N,N*-dimethylethanolamine; PC, phosphatidylcholine. In each case the 1,2-dipalmitoyl derivative was the one examined (contamination with other fatty acyl chains < 0.5%).

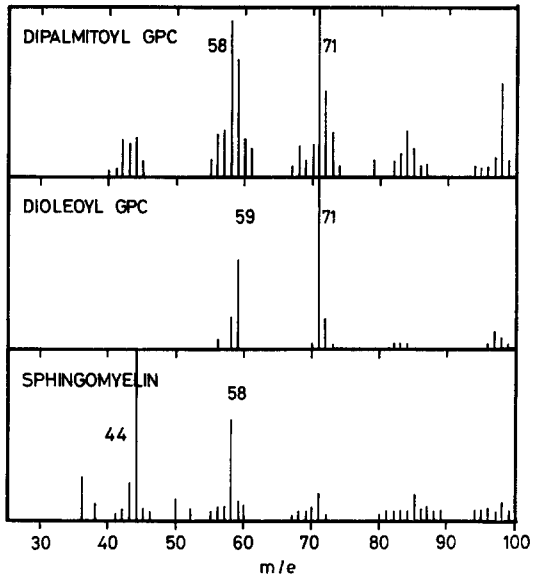


FIG. 2. Mass spectra for three compounds containing the phosphorylcholine group, recorded at 14 ev, showing *m/e* values between 25 and 100. Intensities are relative to the largest peak in this region. Peaks smaller than 1% are not shown.

orders of magnitude greater than the subsequent fragmentations of the P^+ ion. If there were sufficient information about the above reactions it would be possible to estimate these rate constants by application of the quasi-equilibrium theory (QET) (9, 31), using the appearance potential data already reported (10).

Partial mass spectra between *m/e* 25 and 100 for various phosphatidyl amino alcohols, recorded at an electron voltage of 14 ev, are shown in Fig. 1. In each case the acyl group was made up of palmitoyl chains only. Comparable data are shown in Fig. 2 for three compounds containing the *O*-phosphorylated choline group, one of which (sphingomyelin) possesses a second nitrogen atom. The sphingomyelin was obtained from ox brain and had the following fatty acid composition by weight: $C_{16:0}$, 2.0%; $C_{18:0}$, 28.4%; $C_{19:0}$, 1.2%; $C_{22:0}$, 2.4%; $C_{23:0}$, 2.1%; $C_{24:0}$, 6.4%; $C_{24:1}$, 32.8%; $C_{25:0}$, 9.4%; $C_{26:0}$, 5.7%; and total unidentified, 9.6%.

Duncan et al. (12) have described mass spectra for the TMS derivatives of deacylated glycerophospholipids, and Cicero and Sherman (32) have reported similar data for the TMS derivatives of deacylated cardiolipin.

Accurate mass measurements indicate that typical nitrogen-containing ions in the spectra of the phosphatidyl amino alcohols have the following ionic formulas:

	calculated mass	*measured mass*
CH_4N	30.0344	30.0350
C_2H_6N	43.0422	43.0419
C_3H_8N	58.0657	58.0657
C_3H_9N	59.0735	59.0751
C_4H_9N	71.0735	71.0732

A peak at *m/e* 98 ($C_6H_{10}O$) has been reported previously (7) and is present in the spectra of all the 1,2-dipalmitoyl derivatives studied, including 1,2-dipalmitoyl glycerophosphate (phosphatidic acid). The peak at *m/e* 84 (measured mass, 84.0576; calculated for C_5H_8O, 84.0575) is the lower homolog of this ion.

The ion at *m/e* 85 in the spectrum of dipalmitoyl GPE cannot have arisen from the intact molecule by a simple fragmentation reaction, since accurate mass measurements establish that this ion has the composition C_4H_7NO (measured mass, 85.0534; calculated mass, 85.0528). It is unlikely to be an artifact because it has been observed with two different samples of dipalmitoyl GPE on a number of separate occasions. This ion may be produced by either an ion–molecule reaction (33), which is unlikely because of the low source and analyzer pressures, or more probably by expulsion of the phosphorus atom (7, 34, 35), as this phenomenon has been reported previously for other phosphate esters. This ion has also been observed in the low voltage spectrum of ceramidyl ethanolamine.[1]

The peaks at *m/e* 30, 44, and 58 are typical of the fragmentations seen with substituted aliphatic amines (36, 37). The ratio of intensities for the ion pair *m/e* 58/59 in the spectra of choline-containing compounds differs from compound to compound. This point is illustrated in Fig. 2, and one must suspect that the choline moiety is undergoing some form of thermal elimination reaction in the source region of the mass spectrometer, since these reactions are known to occur with quaternary nitrogen compounds (38–40).

At this stage, no absolute criteria can be established for the interpretation of the spectra shown in Figs. 1 and 2, with the exception of the ion at *m/e* 30, which is indicative of a primary amino group. Also, the possibility of thermal reactions must be considered, particularly when dealing with small fragments at the low-mass end of the spectrum.

It has been demonstrated by Perkins and Johnston (13) and by Horning et al. (14) that GLC may be used to study the diacyl glycerol portion of phospholipids after elimination of the phosphate group. Horning et al. (14) formed the trimethylsilyl derivatives of the diglycerides and showed by mass spectrometry that these were the *O*-trimethylsilyl compounds. The procedure used for the elimination of the phosphate group resulted in variable isomerization of the 1,2-diglycerides to the 1,3 isomers.

The spectra reported in this paper (Fig. 3) demonstrate that it is possible to detect fragments related to the molecular species of phosphatidylcholines in naturally occurring mixtures. Phosphatidylcholine fractions

[1] Klein, R. A., and P. Kemp. Unpublished data.

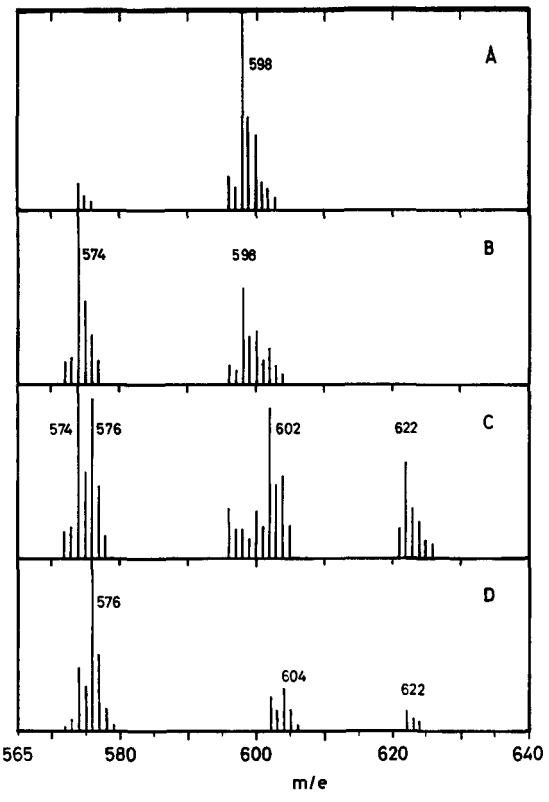


FIG. 3. Mass spectra for phosphatidylcholine fractions A–D, recorded at 70 ev, showing *m/e* values between 565 and 640. The appropriate fatty acid compositions are listed in Table 3. Intensities are relative to the largest peak in this region of the spectrum. Peaks less than 1% relative abundance are not shown.

A–D were derived from natural sources as described in Methods. A GLC analysis of the constituent fatty acids and the positional distribution of these acids has been performed using phospholipase A_2 (EC 3.1.1.4) from *Naja naja* venom (25), and the results are tabulated below as the mole percentage of each fatty acid methyl ester (Table 3).

TABLE 3. Positional analysis of phosphatidylcholine fractions A–D by GLC

Fatty Acid	Fraction A		Fraction B		Fraction C		Fraction D	
	α	β	α	β	α	β	α	β
16:0[a]	6.24	1.38	41.84	3.61	65.06	0.80	70.92	0.63
18:0	3.25	0.32	7.43	0.41	27.40	0.30	23.37	n.d.
18:1	9.56	8.68	8.03	11.43	6.61	15.14	4.11	42.67
18:2	72.62	80.44	38.85	78.28	n.d.	11.64	n.d.	15.06
18:3	8.33	9.18	3.85	6.26	n.d.	n.d.	tr	n.d.
20:4					n.d.	3.68	n.d.	2.00
20:5					n.d.	15.65	n.d.	7.48
22:5					n.d.	5.08	n.d.	2.83
22:6					n.d.	46.70	n.d.	27.74

Results expressed as mole % of fatty acid in the α and β positions as determined by selective hydrolysis with phospholipase A_2 from *Naja naja* venom. n.d., not detected; tr, trace amount. Only values greater than 1% are shown, except for those components which are present at higher concentration in another position or fraction.

[a] Number of carbon atoms:number of double bonds.

TABLE 4. Major ions in the mass spectra of the phosphatidylcholine fractions A–D related to the number of carbon atoms and double bonds in the acyl side chains

m/e	Number of Carbon Atoms:Number of Double Bonds	Probable Identity
574	34:2	Palmitoyl-linoleoyl[a]
576	34:1	Palmitoyl-oleoyl[a]
596	36:5	Palmitoyl-eicosa-pentaenoyl
598	36:4	Dilinoleoyl
600	36:3	Oleoyl-linoleoyl[b]
602	36:2	Stearoyl-linoleoyl[c]
604	36:1	Stearoyl-oleoyl[a]
622	38:6	Palmitoyl-docosa-hexaenoyl

The probable identity of each ion is based on the fatty acid positional analysis for each fraction.

[a] Compare Horning et al. (14).

[b] This ion could also be stearoyl-linolenoyl, but there is insufficient 18:3 in fractions C and D for this to be likely.

[c] When the combination of fatty acids is dioleoyl, this ion has a mass of *m/e* 603 (7).

The spectra in Fig. 3 exhibit groups of ions spaced by 28 mass units, with a 2-unit spacing within each group. The actual mass of the ion identifies (*a*) the number of carbon atoms in the diglyceride fragment and (*b*) the number of double bonds in the two fatty acid chains. For instance, *m/e* 604 indicates a total of 36 side-chain carbon atoms and the presence of a single double bond. It has been shown previously that stearoyl-oleoyl GPC yields an ion at *m/e* 604 (7). The molecular species may be tentatively identified from a consideration of the fatty acid composition of each fraction, and these identifications are shown in Table 4.

If one compares the relative peak heights in the mass spectra with the estimated species composition (on a random basis) from the fatty acid positional analysis of the various fractions, it is apparent that the species containing the smallest number of carbon atoms give ion intensities that are greater than would be expected. The parameters affecting the observed ion intensity have been discussed recently with regard to the mass spectra of mixed triglycerides by Hites (41), who derived correction factors for the various isomeric triglycerides containing palmitoyl and stearoyl chains and showed that these obeyed a log-linear relationship against carbon number (42). Hites (41) questioned the use of formal correction factors on two counts: first, that since ". . . .structurally different triglycerides are represented by the same mass. . .there can be no unique correction factor"; and second, he questioned the validity of the correction factor used for glycerides containing one or more double bonds.

Ion intensities will be affected as well by the relative stability of the ions. Those containing polyunsaturated

side chains would be the least stable under electron impact (43). If all polyunsaturated chains were to be fully deuterated, it should be possible to distinguish the number of double bonds originally present, and the chains should have comparable stabilities. This reduction was attempted using fully deuterated hydrazine, prepared from anhydrous hydrazine by D–H exchange (44, 45). Considerable quantities of lysophosphatidylcholine were formed, however, and the reaction was only partially complete after 48 hr. Although catalytic reduction with deuterium gas and platinum oxide catalyst (46) proceeded smoothly with little degradation of the phosphatidylcholine, considerable D–H scrambling occurred (47), rendering this method unsuitable also.

An attempt was made, therefore, to determine the molar correction factors for those phosphatidylcholines that were available as a result of chemical synthesis. These factors were obtained using phosphatidylcholine mixtures of known molar ratio and comparing this ratio with the ratio of relative ion abundances at 70 ev, with dipalmitoyl GPC as an internal standard. Factors obtained at low electron voltage were indistinguishable experimentally from those obtained at 70 ev. The molar correction factors derived in this manner are shown in Table 5. These results are similar to those of Hites (41) in that the response was greatest for those compounds containing the smallest number of carbon atoms and the introduction of one double bond increased the response approximately twofold.

In order to use this technique for investigating phosphatidylcholine molecular species (and by extension, other phosphatidyl bases) in biological tissues on a quantitative basis, it would be necessary to obtain correction factors for the commonly occurring species and, in certain cases, for the less readily available molecular species if the tissue were rich in a particular fatty acid.

TABLE 5. Molar correction factors for various 1,2-diacyl glycerylphosphorylcholines[a]

1,2-Diacyl GPC	Molar Correction Factor
Dimyristoyl	1.75 ± 0.06
Dipalmitoyl	1.00[b]
Distearoyl	0.70 ± 0.02
Dioleoyl	0.92 ± 0.01
(1-Stearoyl-2-oleoyl)	1.48 ± 0.10
(1-Oleoyl-2-stearoyl)	1.32 ± 0.14

Molar correction factor is defined as the relative ion intensity, with respect to dipalmitoyl GPC, for an equivalent molar quantity of each diacyl GPC.

[a] When estimated by the intensity of the ion corresponding to the diglyceride moiety in an AEI MS902 mass spectrometer with a source temperature of 250°C and an electron voltage of 70 ev.

[b] By definition.

The results presented in this and previous papers (7, 10) show that it is possible to investigate the structure of phospholipids by mass spectrometry even though these compounds are comparatively nonvolatile and the molecular ions have rather short lifetimes. In particular, "nonapparent" ions may be detected by metastable scanning in the 1FFR. Appearance potentials and accurate mass measurements provide additional mechanistic and structural information. Fragments arising from the amino alcohol moiety have been identified in low voltage spectra by accurate mass measurement, and the measurement of ion intensities in the region *m/e* 500–700 has permitted the detection and identification of fragments directly related to the molecular species present in a mixture.

All of these techniques may be performed on the purified, but otherwise untreated, intact molecule with as little as 50 μg deposited on the direct insertion probe.

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