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Mass Spectrometric Analyses of Biologically Active Choline Phospholipids and Their Lyso Derivatives

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The electron impact and chemical ionization mass spectra of 1-*O*-hexadecyl- and 1-palmitoyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholines and their lyso derivatives were measured by insertion of the compounds into a direct inlet system. The lysophospholipids were decomposed by heating at over 300 °C into multiple compounds that volatilized together and gave several characteristic ion peaks when subjected to electron impact or interaction with an ion plasma of reactant gas. The mass spectral data indicated that the major pyrolysis products of these lysophospholipids were produced by elimination of methanol or *N,N*-dimethylethanolamine. When *sn*-2-acetyl phospholipids were introduced on the direct insertion probe and heated, several pyrolysis products volatilized together at above 350 °C. The results suggested the major pyrolysis mechanism was loss of the phosphorylcholine moiety, together with some deacetylation and subsequent elimination of methanol and *N,N*-dimethylethanolamine from the *sn*-2-acetyl phospholipids.

Keywords——platelet-activating factor; lysophosphatidylcholine; phospholipid pyrolysis; electron impact mass spectrometry; chemical ionization mass spectrometry

Platelet-activating factor (PAF) derived from antigen-stimulated, IgE-sensitized rabbit basophils was recently shown to be 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine.^{1,2)} This unique phospholipid had a wide variety of strong biological effects.³⁾ Recent studies revealed that the activities of PAF were mainly due to the *O*-alkyl residue at the *sn*-1-position and the *O*-acetyl at the *sn*-2-position of its glycerol moiety; 1-acyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholines were less active while 1-*O*-alkyl and 1-acyl-2-lyso-*sn*-glycero-3-phosphocholines were essentially inactive.¹⁻⁶⁾

It is known that intact glycerophospholipids and their trimethylsilyl (TMS) derivatives are not amenable to gas phase analysis owing to their susceptibilities to thermal degradation. Recently, Polonsky *et al.*⁷⁾ have demonstrated that chemical ionization mass spectrometry with a direct inlet system was applicable to structural determination of non-derivatized PAF and its lyso derivatives using isobutane as a reactant gas; a protonated molecular ion and other characteristic fragment ions of significant diagnostic value were detected in these spectra and possible structures of the ions were presented. However, the fragmentation mechanisms of these phospholipids have not been fully interpreted. We previously reported that biologically active lysophosphatidic acids (LPAs) and their dimethyl esters were degraded on the direct insertion probe during heating into pyrolysis products retaining phosphorus in the molecules, and that these pyrolysis products vaporized, yielding characteristic ions of diagnostic value on electron impact and chemical ionization. Among the techniques used in that study, high resolution mass spectrometry and ammonia chemical ionization mass spectrometry were very useful for clarification of the pyrolytic mechanism of these phospholipids. Therefore, we examined the mass spectra of the choline phospholipids using different

techniques in order to elucidate whether they could escape thermal degradation in a direct inlet system. Furthermore, we wished to examine whether these active choline phospholipids could be distinguished from their lyso derivatives by electron impact or chemical ionization mass spectrometry, because the former obtained from natural sources are often contaminated by the latter owing to the similarity of their chromatographic behaviors.

Experimental

Phospholipids—1-Palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine (16:0-lyso-PC) was purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). 1-*O*-Hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine (16:0-lyso-PAF) and 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (16:0-PAF) were obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland). 1-Palmitoyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (16:0—2:0-PC) was prepared as follows. A sample of 20 mg of 16:0-lyso-PC was dissolved in 5 ml of benzene-chloroform-acetonitrile mixture (2:1:2, by vol.). Acetic anhydride (0.5 ml) and 4-dimethylaminopyridine (40 mg) were added, and the mixture was heated at above 60 °C for 2 h with vigorous stirring. After confirmation that the acetylation reaction was complete, 5 ml of methanol was added and the solvents were evaporated off under reduced pressure. The residue was dissolved with 20 ml of chloroform-methanol mixture (2:1, v/v) and the extract was washed three times with 5 ml of 0.1 N HCl to remove 4-dimethylaminopyridine. The preparation of 16:0—2:0-PC gave a single spot on silica gel with chloroform-methanol-water (65:35:5, by vol.) as the solvent.

Mass Spectrometric Measurements—Electron impact and chemical ionization mass spectra (EI-MS/CI-MS) were measured with a JEOL JMS-D 300 double focussing mass spectrometer with a direct inlet system. The standard conditions for EI mass spectrometry were as follows: ionization energy, 20 eV; ionization current, 300 μ A; accelerating voltage, 3.0 kV; temperature of ion source, 250 °C. High resolution mass spectra were obtained with perfluorokerosene as a standard. CI-MS were obtained under the following conditions: ionization energy, 200 eV; ionization current, 300 μ A; accelerating voltage, 3.0 kV; ion source temperature, 250 °C; pressure of reactant gas (ammonia, isobutane or methane), 1.0 Torr.

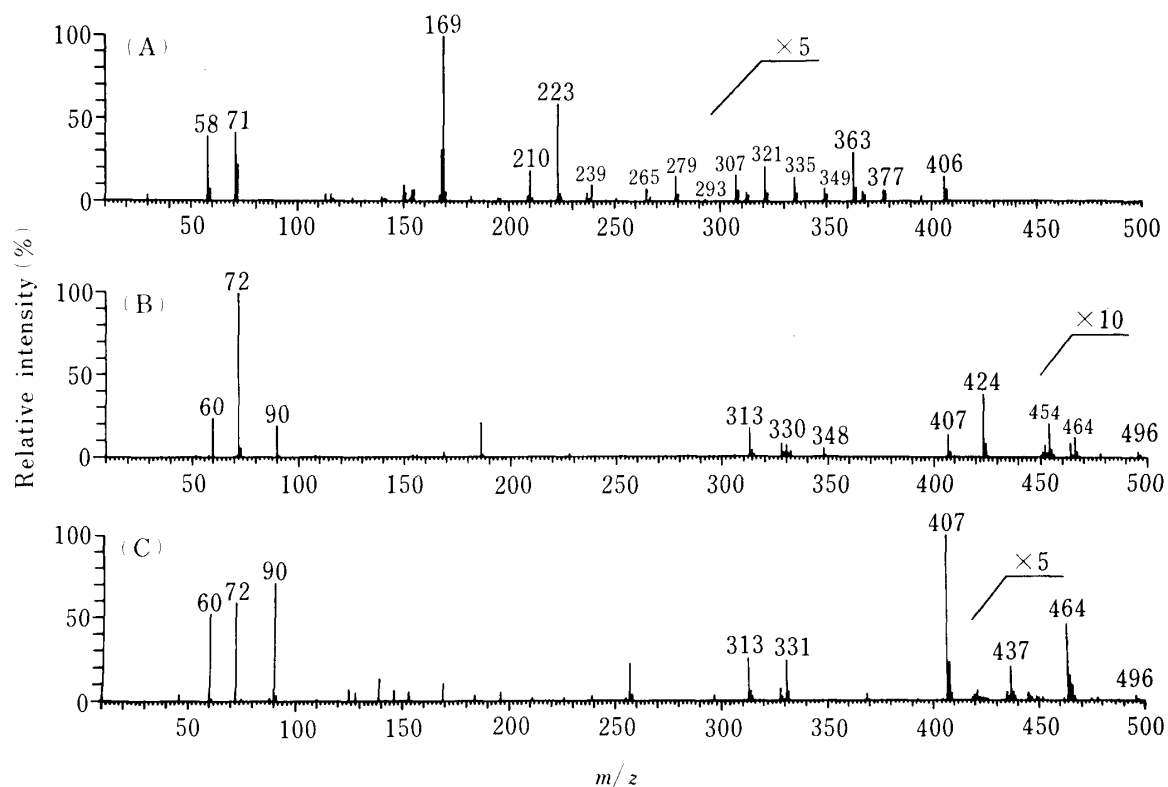


Fig. 1. Mass Spectra of 16:0-Lyso-PC
(A) EI-MS; (B) ammonia CI-MS; (C) isobutane CI-MS.

Results and Discussion

It was found that the *sn*-2-acetyl phospholipids and their lyso derivatives were degraded to several compounds on the heated probe at over 350 and 300 °C, respectively. Thus the observed mass spectra were those of the mixed pyrolysis products as described below.

Mass Spectra of 16:0-Lyso-PC

A typical EI-MS of 16:0-lyso-PC is shown in Fig. 1A. No molecular ion of 16:0-lyso-PC was detected. The ion peak with the highest mass number was at m/z 406. This ion was found to have the formula $C_{20}H_{39}O_6P$ by high-resolution mass spectrometry (data not shown). A homologous series of ion peaks were observed at m/z 223, 237, 251, 265, 279, 293, 307, 321, 335, 349, 363 and 377; these were shown to be species with the general formula $C_nH_{2n-2}O_6P$ by high-resolution mass spectrometry (data not shown). The base peak at m/z 169 and the intense peak at m/z 210 were found to be due to ions with formulae $C_4H_{10}O_5P$ and $C_6H_{11}O_6P$, respectively. The peaks of these phosphorus-containing ions were all the same as those observed in the EI-MS of the dimethyl ester of 1-palmitoyl-lysophosphatidic acid (DM-LPA).⁸⁾ A previous study suggested that DM-LPA was degraded to the cyclic form on the heated probe by the elimination of methanol from the molecule.⁸⁾ Thus, 16:0-lyso-PC might also be converted to the same pyrolysis product with loss of *N,N*-dimethylethanolamine after transposition of one of the *N*-methyl groups of the choline moiety to the phosphate portion as illustrated in Chart 1. If so, the ion at m/z 406 would be the molecular ion of this cyclic

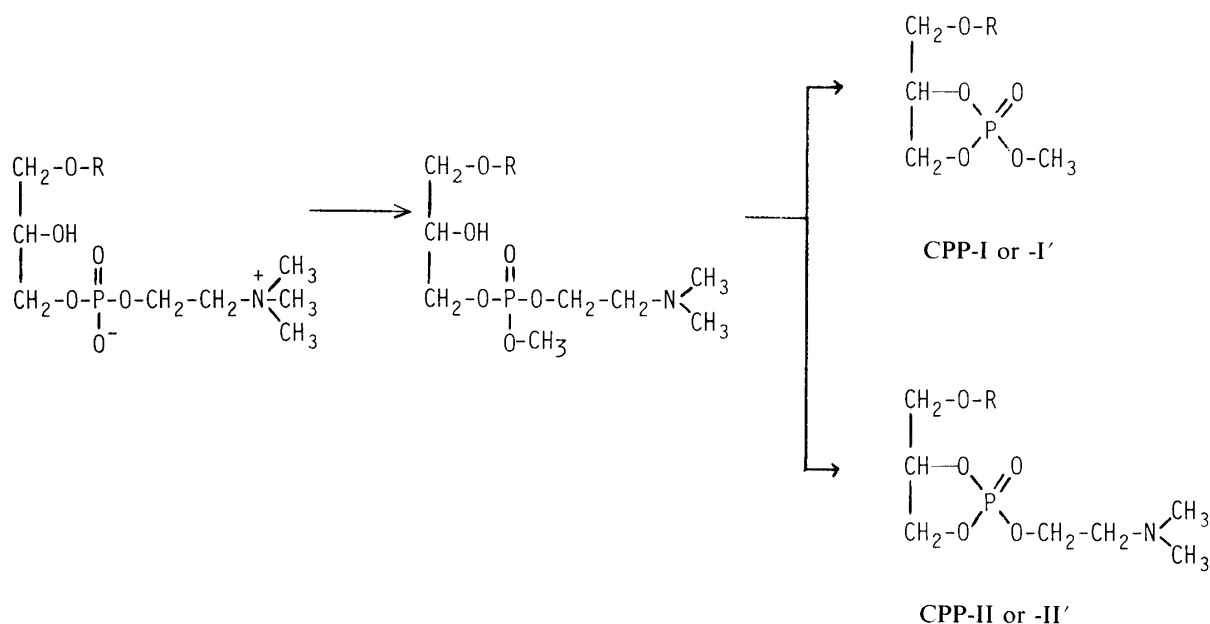
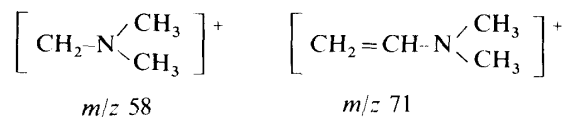


Chart 1. Major Pyrolysis Pathways for 16:0-Lyso-PC and 16:0-Lyso-PAF

CPP-I and -II, 16:0-lyso-PC [$R = CO(CH_2)_{14}CH_3$]; CPP-I' and -II', 16:0-lyso-PAF [$R = (CH_2)_{15}CH_3$].

pyrolysis product (CPP-I), and a homologous series of ions with the general formula $C_nH_{2n-2}O_6P$ would be produced by successive cleavage of the carbon-carbon bonds of its acyl moiety by electron impact. Deacylation of CPP-I yielded the base peak at m/z 169. The fragmentation pattern of 16:0-lyso-PC is similar to that of DM-LPA, but there are two characteristic low-mass ions (m/z 58 and 71) in its EI-MS. High resolution mass spectrometric analysis revealed that these ion peaks were due to species with the formulae C_3H_8N and C_4H_9N , respectively, suggesting that they are derived from the choline moiety of 16:0-lyso-

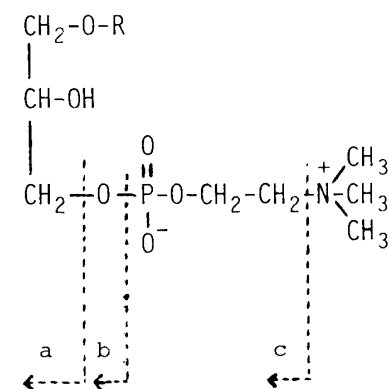
PC. Possible structures are shown below.



To distinguish fragmentation by electron impact from fragmentation due to pyrolysis, the ion-current profiles of extracted ions of the EI-MS of 16:0-lyso-PC were examined. Phosphorus-containing ions having the same profiles were all chromatographically different from the ions at m/z 58 and 71. These findings indicate that the observed mass spectra are due to mixtures of at least two pyrolysis products; namely, on electron impact, various phosphorus-containing ions were formed from CPP-I and some nitrogen-containing ions from the pyrolysis products obtained from the polar head group of 16:0-lyso-PC.

Chemical ionization mass spectrometric analyses of lyso-PC supported the occurrence of thermal degradation of lyso-PC on the probe. When ammonia was used as the reactant gas, ion peaks were observed at m/z 407 and 424, as shown in Fig. 1B. The ion peak at m/z 407 was predominant, and that at m/z 424 was not detected in the isobutane (Fig. 1C) or methane CI-MS (data not shown). These results, together with the previous experimental data for DM-LPA,⁸⁾ suggest that 16:0-lyso-PC, like DM-LPA, was converted to CPP-I during heating, and that the CPP-I interacted with protons and ammonium ions. Thus, the ions at m/z 407 and 424 could be assigned to $[\text{M}-N,N\text{-dimethylethanolamine}] \cdot \text{H}^+$ and $[\text{M}-N,N\text{-dimethylethanolamine}] \cdot \text{NH}_4^+$, respectively. Characteristic ions at m/z 60, 72 and 90 were observed in the ammonia, isobutane and methane CI-MS of 16:0-lyso-PC, and these could be assigned to be $[\text{C}_3\text{H}_9\text{N}] \cdot \text{H}^+$, $[\text{C}_4\text{H}_9\text{N}] \cdot \text{H}^+$ and $[N,N\text{-dimethylethanolamine}] \cdot \text{H}^+$, respectively.

Some high-mass ions were detected in very small amounts both in the ammonia CI-MS (m/z 454, 464 and 496) and isobutane CI-MS (m/z 437, 464 and 496). The ion at m/z 496 is the protonated molecular ion of 16:0-lyso-PC, suggesting that a very small percentage of 16:0-lyso-PC escaped thermal degradation. The ions at m/z 464 in these spectra can be ascribed to $[\text{M}-\text{CH}_3\text{OH}] \cdot \text{H}^+$. Therefore, another pyrolysis product (CPP-II) may be formed besides CPP-I. CPP-II may be produced by cyclization between the hydroxyl group at the *sn*-2-position of the glycerol moiety and the methoxy group transferred to the phosphate portion from the choline moiety as shown in Chart 1. The ions at m/z 437 and 454 were assignable to $[\text{M}-(\text{CH}_3)_3\text{N}] \cdot \text{H}^+$, $[\text{M}-(\text{CH}_3)_3\text{N}] \cdot \text{NH}_4^+$, respectively, as shown in Chart 2. Sugnaux and Djerassi⁹⁾ reported an intense ion ($\text{MH}^+ - 42$) in the desorption CI-MS of dimyristoylphosphatidylcholine and suggested that this ion was produced by replacement of the tri-



compound A or A': cleavage a-H
compound B or B': cleavage b+H
compound C or C': cleavage c

Chart 2. Minor Pyrolysis Pathways for 16:0-Lyso-PC and 16:0-Lyso-PAF

16:0-lyso-PC, $\text{R} = \text{CO}(\text{CH}_2)_{14}\text{CH}_3$; 16:0-lyso-PAF, $\text{R} = (\text{CH}_2)_{15}\text{CH}_3$.

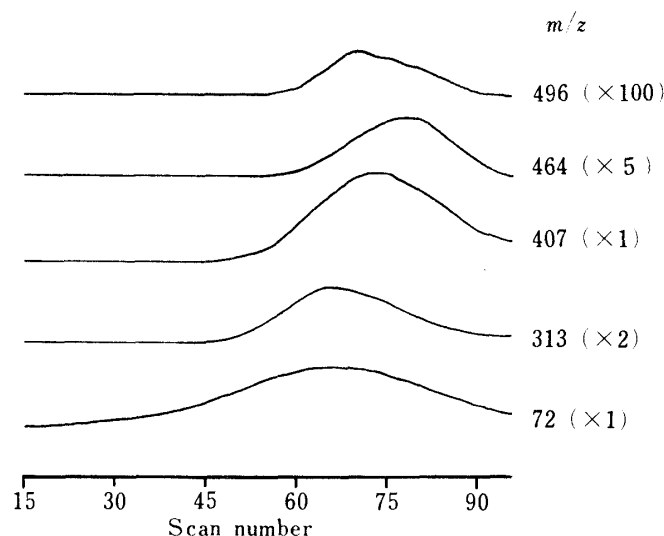


Fig. 2. Ion-Current Profiles of Extracted Ions in the Isobutane CI-MS of 16:0-Lyso-PC

methyamino group in the phosphatidylcholine by ammonia. Because the intensities of these ions (m/z 437 and 454) were low in the CI-MS of 16:0-lyso-PC, elimination of the trimethylamino group was not the major pyrolysis mechanism for this lyso-type choline phospholipid. In addition, a small part of 16:0-lyso-PC seemed to be decomposed to compound A and compound B by loss of the phosphorylcholine moiety, as shown in Chart 2, because weak peaks were seen at m/z 313 ([compound A]·H⁺), 330 ([compound A]·NH₄⁺) and 348 ([compound B]·NH₄⁺) in the ammonia CI-MS, and at m/z 313 ([compound A]·H⁺) and 331 ([compound B]·H⁺) in the methane and isobutane CI-MS.

Figure 2 shows typical tracings of the ion-current profiles in isobutane CI-MS of the extracted ions, which were derived from various pyrolysis products of 16:0-lyso-PC. The profiles of these ions were similar, but not identical. Thus, a large percentage of 16:0-lyso-PC was converted to CPP-I on the heated probe, and this product was present together with other pyrolysis products and intact 16:0-lyso-PC. Sugnaux and Djerassi⁹⁾ have also noted that the desorption CI-MS of dimyristoyl-phosphatidylcholine differed to some extent from scan to scan; desorption of intact neutral molecules took place for a short period, immediately followed by desorption of decomposition products formed on the surface.

Mass Spectra of 16:0-Lyso-PAF

An example of the EI-MS is given in Fig. 3A. It showed no molecular ion and displayed few high-mass ions of significant diagnostic value, unlike the EI-MS of 16:0-lyso-PC. Several series of intense low-mass ions were found by high resolution mass spectrometry to be derived from the alkyl chain (data not shown): these ions had the formulae C_nH_{2n+1} , C_nH_{2n} and C_nH_{2n-1} . The strong ions at m/z 58 and 71 were found to have the formulae C_3H_8N and C_4H_9N , respectively, by high-resolution mass spectrometry (Table I), indicating the elimination of the polar head group of 16:0-lyso-PAF like that of 16:0-lyso-PC during heating. The weak peak at m/z 392 was assigned to the species $C_{20}H_{41}O_5P$ by high resolution mass spectrometry (Table I), and this would correspond to the ion at m/z 406 in the EI-MS of 16:0-lyso-PC. Possibly, 16:0-lyso-PAF was degraded to a cyclic pyrolysis product (CPP-I') that was similar to CPP-I from 16:0-lyso-PC as shown in Chart 1. Successive cleavage of the alkyl chain of CPP-I' by electron impact would yield several fragment ions having the formula $C_nH_{2n}O_5P$, as listed in Table I. However, the intensities of these phosphorus-containing fragment ions were far less than those of the corresponding ions derived from 16:0-lyso-PC.

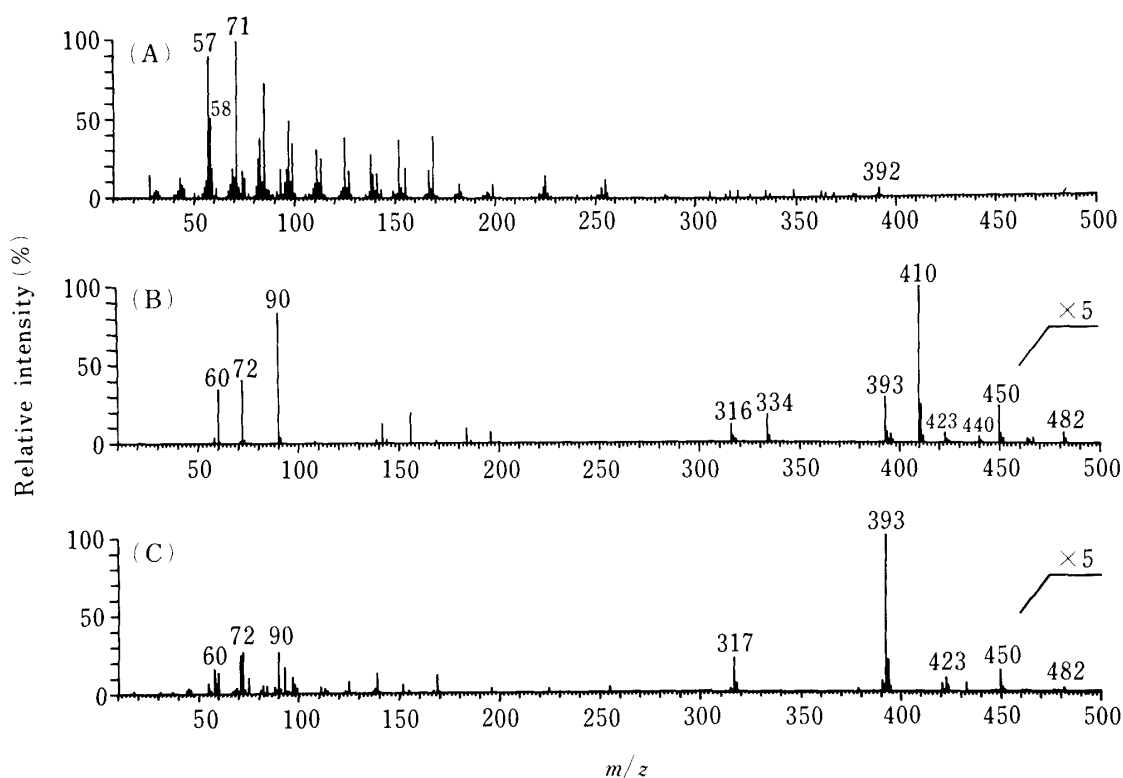


Fig. 3. Mass Spectra of 16:0-Lyso-PAF

(A) EI-MS; (B) ammonia CI-MS; (C) isobutane CI-MS.

Rather, intense low-mass ions associated with the phosphate moiety were observed at m/z 113, 125, 138 and 152. High-resolution mass spectrometric data for these ions are shown in Table I.

Analysis of 16:0-lyso-PC by CI mass spectrometry provided information on the pyrolysis pattern. The ammonia and isobutane CI-MS are shown in Fig. 3B and C, respectively. These spectra show a protonated molecular ion of very low abundance and the expected ion peaks. When ammonia was used as the reactant gas, the ions at m/z 60 ($[\text{C}_3\text{H}_9\text{N}]\cdot\text{H}^+$), 72 ($[\text{C}_4\text{H}_9\text{N}]\cdot\text{H}^+$), 90 ($[\text{N,N-dimethylethanolamine}]\cdot\text{H}^+$), 393 ($[\text{M}-\text{N,N-dimethylethanolamine}]\cdot\text{H}^+$) and 410 ($[\text{M}-\text{N,N-dimethylethanolamine}]\cdot\text{NH}_4^+$) were predominant, and were accompanied by the ion peaks at m/z 423 ($[\text{M}-(\text{CH}_3)_3\text{N}]\cdot\text{H}^+$), 440 ($[\text{M}-(\text{CH}_3)_3\text{N}]\cdot\text{NH}_4^+$), 450 ($[\text{M}-\text{CH}_3\text{OH}]\cdot\text{H}^+$), 316 ($[\text{compound A}']\cdot\text{NH}_4^+$, Chart 2) and 334 ($[\text{compound B}']\cdot\text{NH}_4^+$, Chart 3). The ion at m/z 393 ($[\text{M}-\text{N,N-dimethylethanolamine}]\cdot\text{H}^+$) was the base peak and no peak was observed at m/z 410 in the methane (data not shown) and isobutane CI-MS (Fig. 3C). In these spectra, diagnostic ion peaks were also seen at m/z 60, 72, 90, 317 ($[\text{compound B}']\cdot\text{H}^+$, Chart 2), 423 ($[\text{M}-(\text{CH}_3)_3\text{N}]\cdot\text{H}^+$) and 450 ($[\text{M}-\text{CH}_3\text{OH}]\cdot\text{H}^+$). The isobutane CI-MS of 16:0-lyso-PAF was essentially the same as that reported by Polonsky *et al.*⁷⁾ In the ammonia CI-MS, however, there were some specific ions which would be produced by interaction of the neutral pyrolysis products from 16:0-lyso-PAF with ammonium ions (m/z 316, 334, 410 and 440). Thus, it is very useful for clarification of the fragmentation mechanisms of this lyso-phospholipid to compare the ammonia CI-MS with the isobutane CI-MS.

The ion-current profiles of the ions at m/z 60, 72, 90, 317, 393, 423, 450 and 482 were different from each other. Similarly, the ion-current profiles of the ions at m/z 60, 72, 90, 316, 334, 410, 423, 450 and 482 in the ammonia CI-MS were different, although two sets of ions (m/z

TABLE I. High-Resolution Mass Spectral Data on Characteristic Fragment Ions in EI-MS of 16:0-Lyso-PAF

m/z	Elemental composition	Observed mass	Calculated mass	Relative intensity (%)
392	C ₂₀ H ₄₁ O ₅ P	392.2677	392.2692	5.5
377	C ₁₉ H ₃₈ O ₅ P	377.2484	377.2457	1.2
363	C ₁₈ H ₃₆ O ₅ P	363.2282	363.2300	3.6
349	C ₁₇ H ₃₄ O ₅ P	349.2138	349.2144	5.4
335	C ₁₆ H ₃₂ O ₅ P	335.1954	335.1987	4.7
321	C ₁₅ H ₃₀ O ₅ P	321.1820	321.1831	3.8
307	C ₁₄ H ₂₈ O ₅ P	307.1716	307.1674	3.9
293	C ₁₃ H ₂₆ O ₅ P	293.1481	293.1518	0.7
279	C ₁₂ H ₂₄ O ₅ P	279.1395	279.1361	0.3
265	C ₁₁ H ₂₂ O ₅ P	265.1248	265.1205	0.5
251	C ₁₀ H ₂₀ O ₅ P	251.1063	251.1048	1.8
237	C ₉ H ₁₈ O ₅ P	237.0920	237.0892	0.1
223	C ₈ H ₁₆ O ₅ P	223.0734	223.0735	0.6
167	C ₄ H ₈ O ₅ P	167.0969	167.0110	0.8
	C ₁₂ H ₂₃	167.1759	167.1799	19.6
152	C ₄ H ₉ O ₄ P	152.0197	152.0238	35.2
	C ₁₁ H ₂₀	152.1529	152.1565	3.2
138	C ₃ H ₇ O ₄ P	138.0080	138.0082	26.2
	C ₁₀ H ₁₈	138.1418	138.1409	2.7
125	C ₂ H ₆ O ₄ P	125.0013	125.0004	20.6
	C ₉ H ₁₇	125.1300	125.1330	18.4
113	CH ₆ O ₄ P	113.0003	113.0004	17.8
	C ₈ H ₁₇	113.1314	113.1330	7.2
72	C ₄ H ₁₀ N	72.0838	72.0813	6.9
71	C ₄ H ₉ N	71.0762	71.0735	64.9
58	C ₃ H ₈ N	58.0627	58.0657	50.2

393 and 410; m/z 423 and 450) had the same vaporization profiles. These results indicated that these ions arose independently from various pyrolysis products of 16:0-lyso-PAF by chemical ionization. For instance, after transposition of one of the *N*-methyl groups to the phosphate moiety as proposed by Polonsky *et al.*,⁷⁾ most of the 16:0-lyso-PAF was degraded to CPP-I' or CPP-II' (Chart 1), possibly owing to the lyso structure. On the other hand, a small part of the 16:0-lyso-PAF was decomposed by some other pyrolysis mechanism such as by loss of the trimethylamino group or phosphorylcholine moiety.

The CI-MS of 16:0-lyso-PAF were essentially similar to those of 16:0-lyso-PC. Therefore, the pyrolysis profiles of these two lyso-type phospholipids seemed to be quite similar, but the EI-MS of 16:0-lyso-PAF was not analogous with the EI-MS of 16:0-lyso-PC. Probably, the fragmentation pattern of CPP-I' from 16:0-lyso-PAF was different from that of CPP-I from 16:0-lyso-PC: CPP-I was fragmented to produce various phosphorus-containing ion peaks (C_{*n*}H_{2*n*-2}O₆P) in preference to low-mass ions from the acyl moiety, whereas electron impact ionization of CPP-I' provided ions from its alkyl chain in much greater amounts than phosphorus-containing ions (C_{*n*}H_{2*n*}O₅P).

Mass Spectra of 16:0—2:0-PC

A typical EI-MS of 16:0—2:0-PC is shown in Fig. 4A. This EI-MS has no molecular ion and fewer diagnostic ions with high mass numbers as compared with the EI-MS of 16:0-lyso-PC. The major peaks were found by high-resolution mass spectrometry to have no phos-

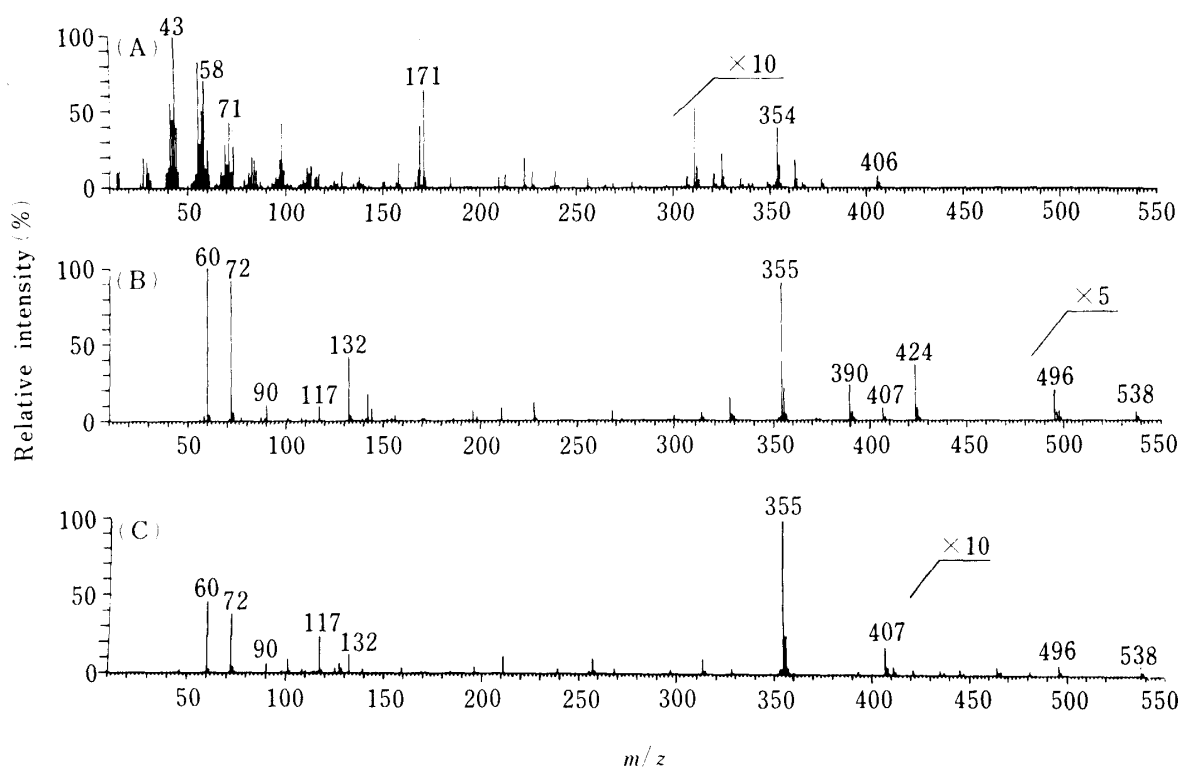
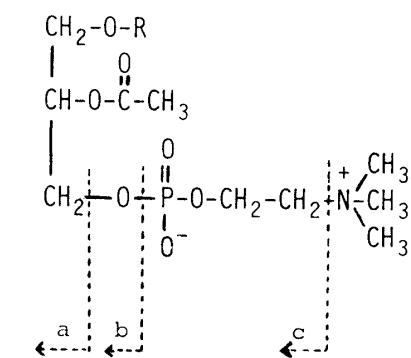


Fig. 4. Mass Spectra of 16:0—2:0-PC
(A) EI-MS; (B) ammonia CI-MS; (C) isobutane CI-MS.

phorus. Some of them had the general formula $C_nH_{2n-5}O_4$ (m/z 339, 325, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 171, 157, 143, 129, 115 and 101). It was evident that some of the low mass ions were derived from the choline moiety (m/z 58 and 71). These observations indicate that elimination of the phosphorylcholine moiety was the primary fragmentation mechanism for 16:0—2:0-PC.

The chemical ionization mass spectrometric analyses¹⁰⁾ supported the interpretation described above and provided further information on the mode of pyrolysis of 16:0—2:0-PC. As shown in Fig. 4B and C, $[M] \cdot H^+$ and $[M-42] \cdot H^+$ were detected in very small amounts in the ammonia and isobutane CI-MS. The isobutane CI-MS showed an intense peak at m/z 355 corresponding to the protonated compound A (Chart 3). Chemical ionization with ammonia provided a characteristic peak at m/z 390 ($[compound\ B] \cdot NH_4^+$, Chart 3) in addition to the ion at m/z 355 ($[compound\ A] \cdot H^+$, Chart 3). These results strongly indicate that most of the 16:0—2:0-PC was degraded to either the corresponding diacylglycerol or its dehydration product by elimination of the phosphorylcholine moiety as shown in Chart 3. The important ions at m/z 407 (isobutane) and m/z 407 and 424 (ammonia) were the same as those originating from the pyrolysis product of 16:0-lyso-PC, CPP-I, indicating that a small amount of 16:0—2:0-PC might be converted to CPP-I by a mechanism similar to that in the case of 16:0-lyso-PC. Because CPP-I seemed to be formed by heating after elimination of the acetyl group of 16:0—2:0-PC, the production of CPP-I may be expected to be less in the case of 16:0—2:0-PC than with 16:0-lyso-PC.

Ions characteristics of choline phospholipids (m/z 60, 72, and 90) were also observed together with the ions at m/z 108, 117 and 132 in the isobutane and ammonia CI-MS of 16:0—2:0-PC. The latter ions could not be detected in the CI-MS of the two lyso-type phospholipids. Conceivably, the formation of these ions may be closely associated with the major pyrolysis mechanism, the production of the diacylglycerol from 16:0—2:0-PC on heating.



compound A or A': cleavage a-H
 compound B or B': cleavage b+H
 compound C or C': cleavage c

Chart 3. Major Pyrolysis Pathways for 16:0—2:0-PC and 16:0-PAF

16:0—2:0-PC, R=CO(CH₂)₁₄CH₃, 16:0-PAF, R=(CH₂)₁₅CH₃.

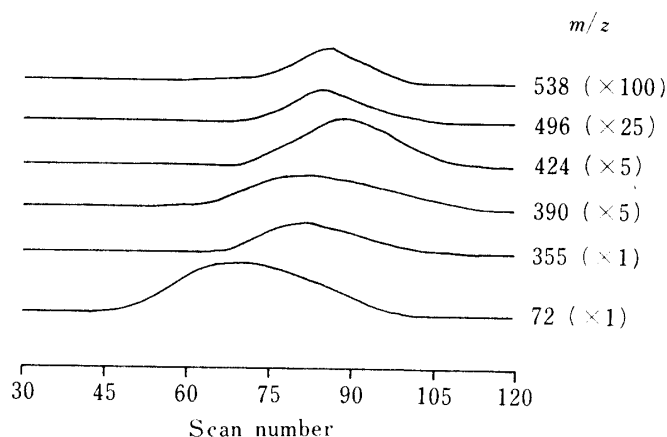


Fig. 5. Ion-Current Profiles of Extracted Ions in the Ammonia CI-MS of 16:0—2:0-PC

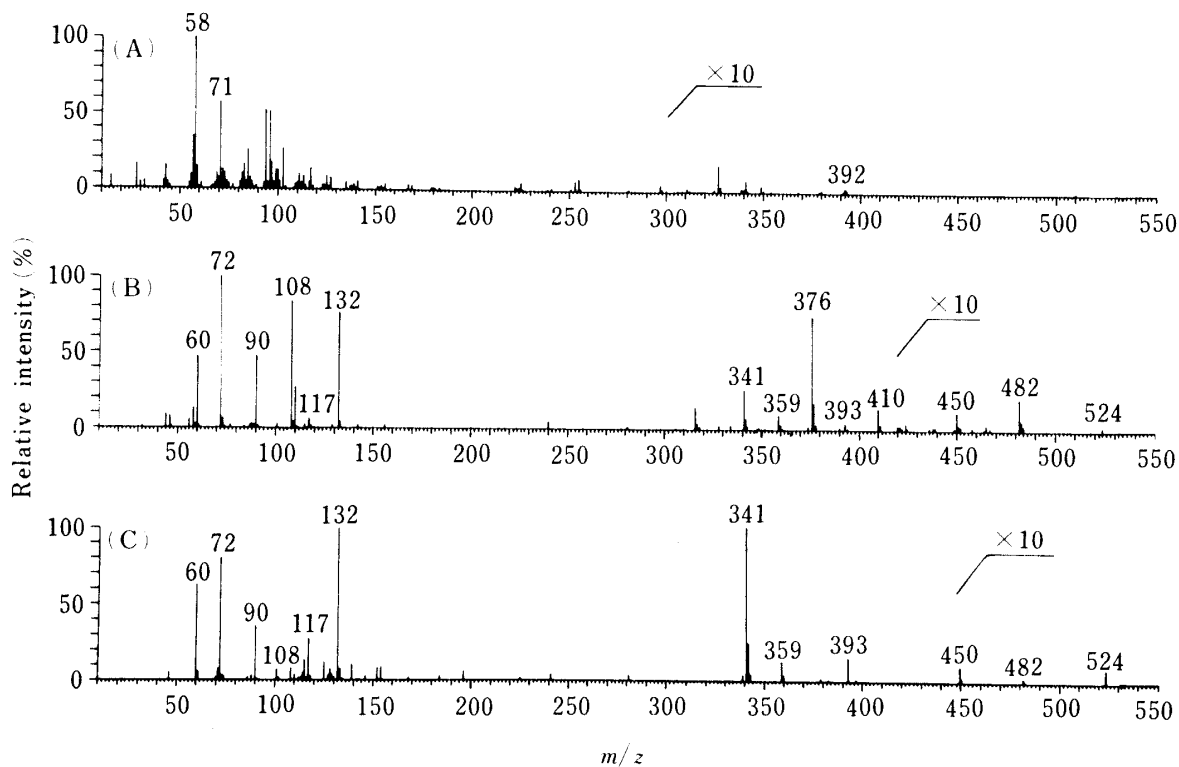


Fig. 6. Mass Spectra of 16:0-PAF

(A) EI-MS; (B) ammonia CI-MS; (C) isobutane CI-MS.

Figure 5 shows typical tracings of selected ion records for the ammonia CI-MS of 16:0—2:0-PC. The ion-current profiles of these representative ions differ from each other, suggesting that they were produced from different pyrolysis products originating from 16:0—2:0-PC with the exception of *m/z* 538 ([M]·H⁺). It was concluded that when 16:0—2:0-PC was introduced on the direct inlet system and heated, it was converted to several pyrolysis products at over 350 °C. Thus, the observed EI- and CI-MS were the overall spectra of these mixed products. In this case, elimination of phosphorylcholine from the molecule may predominate over deacetylation and subsequent cyclization to CPP-I or CPP-II, in contrast to

the cases of 16:0-lyso-PC and 16:0-lyso-PAF.

Mass Spectra of 16:0-PAF

Figure 6A shows a typical EI-MS of 16:0-PAF, which has no molecular ion and few high-mass ions of significant value for structural elucidation. High-resolution mass measurements showed many low-mass ions (m/z 55, 57, 69, 71, 83, 85, 97, 99, 111, 113, 125, 127, 139, 141, 153, 155, 167, 169) as hydrocarbon fragment ions in addition to some intense nitrogen-containing ions at m/z 58 and 71, as in the case of 16:0—2:0-PC. These results suggested that loss of phosphorylcholine was the primary fragmentation mechanism for 16:0-PAF as well as 16:0—2:0-PC.

The interpretation described above was confirmed by examination of the CI-MS of 16:0-PAF.¹⁰⁾ Typical ammonia and isobutane CI-MS are shown in Figure 6B and C. The isobutane CI-MS was essentially the same as that reported by Polonsky *et al.*⁷⁾ Both ammonia and isobutane CI-MS give intense signals at m/z 60, 72, 90, 108, 117 and 132. Possibly, the formation of these ions is associated with the major pyrolysis pathway of 16:0-PAF including loss of the phosphorylcholine moiety, as with 16:0—2:0-PC. Although a protonated ion (m/z 524) and $[M - CH_2 = C = O] \cdot H^+$ (m/z 482) are observed in both spectra, their intensities are low. With isobutane as the reactant gas, the expected ions were detected at m/z 450 ($[M - 42 - CH_3OH] \cdot H^+$), 393 ($[M - 42 - N, N\text{-dimethylethanolamine}] \cdot H^+$), 359 ($[compound\ B'] \cdot H^+$, Chart 3) and 341 ($[compound\ A'] \cdot H^+$, Chart 3). The ions of m/z 450 and 393 would arise from the pyrolysis products of 16:0-PAF, CPP-II' and CPP-I' as illustrated in Chart 1. With ammonia as the reactant gas, characteristic adduct ions at m/z 410 ($[M - 42 - N, N\text{-dimethylethanolamine}] \cdot NH_4^+$) and 376 ($[compound\ B'] \cdot NH_4^+$, Chart 3) were seen together with ions such as m/z 450, 393, 359 and 341.

Additional examinations of the ion-current profiles of these ions confirmed that 16:0-PAF was converted to many breakdown products on the heated probe (data not shown). Thus, as with 16:0—2:0-PC, the major pathway of pyrolysis of 16:0-PAF seemed to start with elimination of the phosphorylcholine moiety, and this was accompanied by minor pathways such as elimination of the acetyl group and subsequent cyclization to CPP-I' and -II'. In addition, a very small amount of 16:0-PAF escaped thermal degradation.

The present study revealed that biologically active 16:0-PAF and 16:0—2:0-PC were decomposed to multiple products on a heated probe at above 350°C, and that these pyrolysis products vaporized together. Thus, the observed mass spectra showed ion peaks corresponding to a mixture of these pyrolysis products. It should be stressed that comparison of the ammonia and isobutane CI-MS was very useful in reaching the conclusions described above, together with high-resolution mass spectrometric measurements. Although the relative intensities of the ion peaks differed somewhat from scan to scan in the mass measurements, the observed spectra, particularly those obtained by CI, provided significant information for structural determination. In addition, because the lyso-type choline phospholipids were found to be degraded by somewhat different pyrolysis mechanisms on the heated probe over 300°C, mass spectrometry by EI and CI can be used to distinguish PAF and its acyl analogs from their lyso derivatives.

References and Notes

- 1) C. A. Demopoulos, R. N. Pinckard and D. J. Hanahan, *J. Biol. Chem.*, **254**, 9355 (1979).
- 2) J. Benveniste, M. Tenc , P. Varenne, J. Bidault, C. Boulet and J. Polonsky, *C. R. Acad. Sci., Ser. D*, **289**, 1037 (1979).
- 3) R. N. Pinckard, L. McManus and D. J. Hanahan, *Adv. Inflammation Res.*, **4**, 147 (1982).
- 4) M. Tenc , E. Coeffier, F. Heymans, J. Polonsky, J.-J. Godfroid and J. Benveniste, *Biochimie*, **63**, 723 (1981).
- 5) D. J. Hanahan, P. G. Munder, K. Satouchi, L. McManus and R. N. Pinckard, *Biochem. Biophys. Res. Commun.*, **99**, 183 (1981).

- 6) R. L. Wykle, C. H. Miller, J. C. Lewis, J. D. Schmitt, J. A. Smith, J. R. Surles, C. Piantadosi and J. T. O'Flaherty, *Biochem. Biophys. Res. Commun.*, **100**, 1651 (1981).
- 7) J. Polonsky, M. Tencé, P. Varenne, B. C. Das, J. Lunel and J. Benveniste, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 7019 (1980).
- 8) A. Tokumura, Y. Handa, Y. Yoshioka, M. Higashimoto and H. Tsukatani, *Chem. Pharm. Bull.*, **30**, 2119 (1982).
- 9) F. R. Sugnaux and C. Djerassi, *J. Chromatogr.*, **251**, 189 (1982).
- 10) Because the methane CI-MS of 16:0-2:0-PC and 16:0—PAF were essentially similar to their isobutane CI-MS, respectively the data for methane CI-MS are not given in this paper.