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Mass Spectrometric Analyses of Lysophosphatidic Acids and Their Dimethyl Esters

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The electron impact and chemical ionization mass spectra of lysophosphatidic acids and their dimethyl esters were measured by injection of the compounds into a direct inlet system. Dry powders of lysophosphatidic acids were dehydrated on the probe at 200—250°C and the dehydrated lysophosphatidic acids were subjected to electron impact or interaction with ion plasma of the reactant gas, yielding several characteristic ion peaks containing phosphorus. On the other hand, lysophosphatidic acids injected in the probe as solutions in organic solvents were not dehydrated in this temperature range, but were dephosphorylated at higher probe temperatures.

When the dimethyl esters were applied to the probe either as powder or solution, they were converted to the monomethyl esters of dehydrated lysophosphatidic acids on the heated probe, possibly by elimination of methanol from the glycerol backbone and polar phosphate portion. The monomethyl esters were also volatilized and subjected to electron impact and chemical ionization, producing ion peaks corresponding to those observed on analysis of dehydrated lysophosphatidic acids.

Keywords——pyrolysis of lysophosphatidic acid; lysophosphatidic acid methyl ester; electron impact mass spectrometry; chemical ionization mass spectrometry

Lysophosphatidic acid (LPA), an intermediate in the *de novo* synthetic pathway of glycerophospholipids,[1] has recently been found to have some biological effects[2–10] that are similar to those of prostaglandins and thromboxanes. For studies on the physiological and pathophysiological roles of LPA, a sensitive and specific method is needed for detecting trace amounts of LPA. Gas chromatography–mass spectrometry, however, has only been applied to analyses of the deacylation products or hydrophobic parts of glycerophospholipids after chemical or enzymatic degradation. In previous attempts to analyze intact phospholipids or their trimethylsilyl derivatives in a gas phase system, the only products detected were diacylglycerols or their trimethylsilyl derivatives,[11–13] possibly owing to the susceptibility of the phosphorus-oxygen bond to thermal degradation. Klein[14,15] reported the mass spectra of phosphatidylcholines using a direct inlet system. Although these spectra resembled those of the corresponding diacyl-glycerols, there were both phosphorus-containing and nitrogen-containing fragment ions derived from the polar head group of the phospholipids. Folz[16] reported the chemical ionization mass spectrum of dioleoyl-phosphatidylcholine, where the $[M+1]^+$ ion had a relative abundance of over 30% of the base peak. These interesting mass spectrometric studies prompted us to analyze LPA by mass spectrometry using direct probe insertion.

Experimental

Preparation of Lysophosphatidic Acids and Their Dimethyl Esters——1-Decanoyl- and 1-palmitoyl-LPA were purchased from Serdary Research Laboratories Inc. (London, Ontario, Canada) and purified by Sephadex LH-20 column chromatography with chloroform–methanol mixture (1: 1, v/v) as a solvent. 1-Lauroyl-, 1-myristoyl- and 1-stearoyl-LPA were prepared from the corresponding synthetic lysolecithins(1-acyl-*sn*-glycero-3-phosphocholines) obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) by treatment with phospholipase D according to the method of Long *et al.*[17] They were also purified by Sephadex LH-20

column chromatography as described above.

The dimethyl esters of LPAs (DM-LPA) were prepared as follows: LPA was dissolved in chloroform–methanol mixture (17: 3, v/v) and ethereal diazomethane was added to this solution until a yellow color persisted. Then the reaction mixture was concentrated under a stream of nitrogen. Deuterated DM-LPA was prepared by methylation of LPA in *N*,*N*-dimethylformamide with iodomethane-d_3 (Merck Sharp Dohm) in the presence of silver oxide.

Mass Spectrometric Measurements——Electron impact and chemical ionization mass spectra were measured with a JEOL JMS-D300 double-focussing mass spectrometer. Samples as solutions in organic solvents or as dry powders were applied to a direct insertion probe. The standard conditions for measurements of electron impact mass spectrometry were as follows: ionization energy, 20 eV; ionization current, 300 μA; accelerating voltage, 3.5 kV; temperature of ion source, 230°C. High resolution mass spectra were obtained with perfluorokerosene as a standard. Chemical ionization mass spectra were obtained under the following conditions: ionization energy, 200 eV; ionization current, 300 μA; accelerating voltage, 3.5 kV; pressure of reactant gas, 1.0 Torr.

Results and Discussion

When dry powder of palmitoyl-LPA was introduced onto the insertion probe and heated, a component volatilized in the temperature range of 200—250°C. The electron impact mass spectrun (EI–MS) of the component is shown in Fig. 1. The ion peak at *m/z* 392 showed the highest mass number in this spectrum and was found to be due to a species with the formula $C_{19}H_{37}O_6P$ by high resolution mass spectrometry (Table I); this species would be produced by dehydration of LPA. A homologous series of peaks of fragment ions having the general formula $C_nH_{2n-2}O_6P$ (*m/z* 209, 223, 237, 251, 265, 279, 293, 307, 321, 335, 349, 363 and 377)

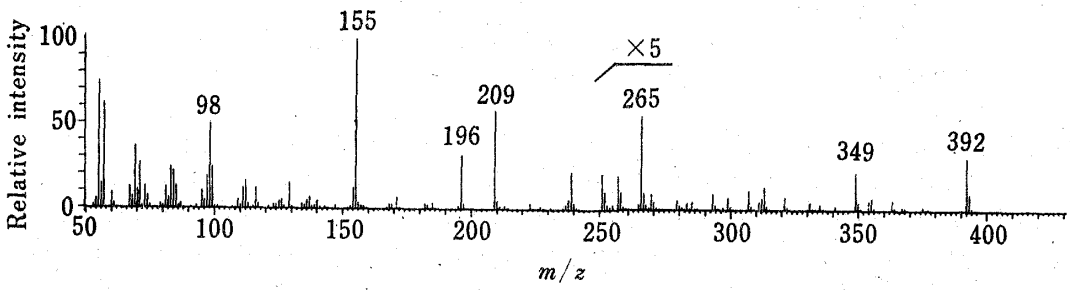

Fig. 1. EI–MS of Dry Powder of Palmitoyl–LPA

TABLE I. High Resolution Mass Spectral Data for Phosphorus-containing Fragment Ions of Dehydrated Palmitoyl–LPA

m/z	Elemental composition	Observed mass	Calculated mass	Relative Intensity (%)
392	$C_{19}H_{37}O_6P$	392.2311	392.2326	6.4
377	$C_{18}H_{34}O_6P$	377.2073	377.2091	0.4
363	$C_{17}H_{32}O_6P$	363.1952	363.1935	1.5
349	$C_{16}H_{30}O_6P$	349.1795	349.1778	5.0
335	$C_{15}H_{28}O_6P$	335.1600	335.1622	0.8
321	$C_{14}H_{26}O_6P$	321.1417	321.1465	1.6
307	$C_{13}H_{24}O_6P$	307.1313	307.1309	2.4
293	$C_{12}H_{22}O_6F$	293.1152	293.1152	2.1
279	$C_{11}H_{20}O_6P$	279.0969	279.0996	1.4
265	$C_{10}H_{18}O_6P$	265.0811	265.0840	10.8
251	$C_9H_{16}O_6P$	251.0681	251.0683	4.4
237	$C_8H_{14}O_6P$	237.0542	237.0527	3.4
223	$C_7H_{12}O_6P$	223.0386	223.0371	4.2
209	$C_6H_{10}O_6P$	209.0248	209.0214	58.0
196	$C_5H_9O_6P$	196.0142	196.0136	33.2
155	$C_3H_8O_5P$	155.0087	155.0108	100.0

were detected in this spectrum, and these are presumably derived from the dehydrated LPA by cleavage of carbon-carbon bonds of its acyl moiety. The intense fragment ion at *m/z* 196 was found to have the formula $C_5H_9O_6P$ by high resolution MS. The base peak at *m/z* 155, having the formula $C_3H_8O_5P$, was probably produced by deacylation of the dehydrated LPA and was assignable to $[(M-H_2O)-RCO+2H]^{+\cdot}$. Other characteristic fragment ions at *m/z* 98, 129, 239, 256 and 313 had the formulae $C_6H_{10}O$, $C_7H_{13}O_2$, $C_{16}H_{31}O$ ($[RCO]^{+\cdot}$), $C_{16}H_{32}O_2$ ($[RCOOH]^{+\cdot}$) and $C_{19}H_{37}O_3$ ($[M-H_2PO_4]^{+\cdot}$), respectively.

When the dimethyl ester of palmitoyl-LPA was analyzed as a powder and as a solution in organic solvent, a single component volatilized at lower temperature than that required for evaporation of dehydrated palmitoyl-LPA. In the EI–MS of the component, shown in Fig. 2, no molecular ion was detected, as in the case of palmitoyl-LPA. The ion peak at *m/z* 406 was found by high resolution mass spectrometric analysis to have the formula $C_{20}H_{39}O_6P$ (Table II),

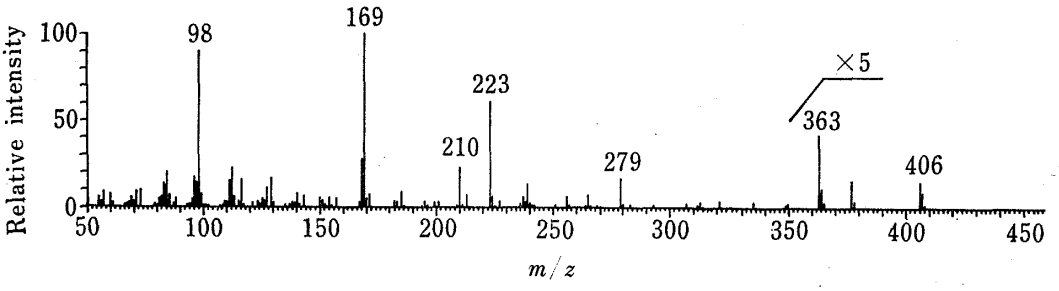

Fig. 2. EI–MS of the Dimethyl Ester of Palmitoyl–LPA

TABLE II. High Resolution Mass Spectral Data for Phosphorus-containing Fragment Ions of the Monomethyl Eater of Dehydrated Palmitoyl–LPA

m/z	Elemental composition	Observed mass	Calculated mass	Relative intensity (%)
406	$C_{20}H_{39}O_6P$	406.2529	406.2483	3.1
377	$C_{18}H_{34}O_6P$	377.2094	377.2091	3.2
363	$C_{17}H_{34}O_6P$	363.1885	363.1935	8.5
349	$C_{16}H_{30}O_6P$	349.1752	349.1778	1.8
335	$C_{15}H_{28}O_6P$	335.1601	335.1622	3.6
321	$C_{14}H_{26}O_6P$	321.1431	321.1465	4.5
307	$C_{13}H_{24}O_6P$	307.1292	307.1309	3.5
293	$C_{12}H_{22}O_6P$	293.1124	293.1153	0.2
279	$C_{11}H_{20}O_6P$	279.1035	279.0996	17.9
265	$C_{10}H_{18}O_6P$	265.0832	265.0840	7.2
251	$C_9H_{16}O_6P$	251.0692	251.0683	0.2
237	$C_8H_{14}O_6P$	237.0582	237.0527	6.8
223	$C_7H_{12}O_6P$	223.0383	223.0371	62.3
210	$C_6H_{11}O_6P$	210.0278	210.0292	23.6
169	$C_4H_{10}O_5P$	169.0206	169.0214	100.0

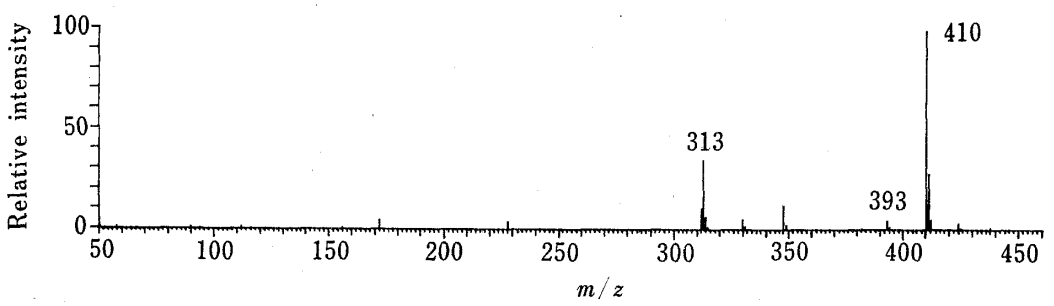

Fig. 3. CI–MS of Dry Powder of Palmitoyl–LPA

indicating that it is probably derived from the dimethyl ester by elimination of methanol. Several characteristic phosphorus-containing fragment ions having the general formula $C_nH_{2n-2}O_6P$ (*m/z* 223, 237, 251, 265, 279, 293, 307, 321, 335, 349, 363 and 377) would be produced by cleavage of carbon-carbon bonds in the acyl moiety of this ion (*m/z* 406). The major fragment ion at *m/z* 210 was found to have the formula $C_6H_{11}O_6P$ by high resolution MS. Complete deacylation of the ion at *m/z* 406 yielded a base peak at *m/z* 169 $[(M-CH_3OH)-RCO+2H]^{+\cdot}$. The other representative fragment ions which do not contain phosphorus were the same as those observed in the spectrum of palmitoyl-LPA. Therefore, the modes of cleavage of palmitoyl-LPA and its dimethyl ester by electron impact seem to be similar.

The chemical ioization mass spectrum (CI–MS) of powder of palmitoyl -LPA was measured with the use of ammonia (Fig. 3), methane and isobutane as reactant gases. The ion peak at *m/z* 393 and the base peak at *m/z* 410 are observed with ammonia; these would be derived from dehydrated LPA by addition of a proton ($[M-H_2O]\cdot H^+$) and an ammonium ion ($[M-H_2O]\cdot NH_4^+$), respectively. The base peaks of palmitoyl-LPA were observed at *m/z* 393 ($[M-H_2O]\cdot H^+$) with methane and isobutane. These results indicate that dehydration of LPA is induced by heating on the probe before reaction with the ion plasma of reactant gas. In the CI–MS of the dimethyl ester of palmitoyl- LPA with ammonia (Fig. 4), the peaks at *m/z* 407 and 424 are major peaks and are possibly produced by addition of a proton and an ammonium ion to the heat-mediated product derived from the dimethyl ester by elimination of methanol ($[M-CH_3OH]\cdot H^+$ and $[M-CH_3OH]\cdot NH_4^+$). The base peaks of the dimethyl ester were observed at *m/z* 407 ($[M-CH_3OH]\cdot H^+$)with methane and isobutane. These results indicate that elimination of methanol from the dimethyl ester is induced by heating on the probe as well as dehydration of LPA. Although it is possible that dehydrated palmitoyl-LPA is formed by elimination of H_2O from the glycerol moiety (structure I and II), it is more likely that palmitoyl-LPA is converted to a cyclic form (structure III) by dehydration between the glycerol moiety and the phosphate ester portion, because its dimethyl ester is converted to a similar structure by elimination of methanol fromthe molecule (Chart 1).

In the EI–MS of the deuterated dimethyl ester of palmitoyl-LPA, *m/z* 406 shifted to *m/z* 409. Other phosphorus-containing fragment ions over *m/z* 223—377, having the formula $C_nH_{2n-2}O_6P$, shifted to ions having the formula $C_nH_{2n-5}D_3O_6P$. The fragment ion at *m/z* 210 and a base peak at *m/z* 169 shifted to *m/z* 213 and 172, respectively. In the CI–MS of the deuterated dimethyl ester, the ions at *m/z* 407 (with methane and isobutane) and at *m/z*

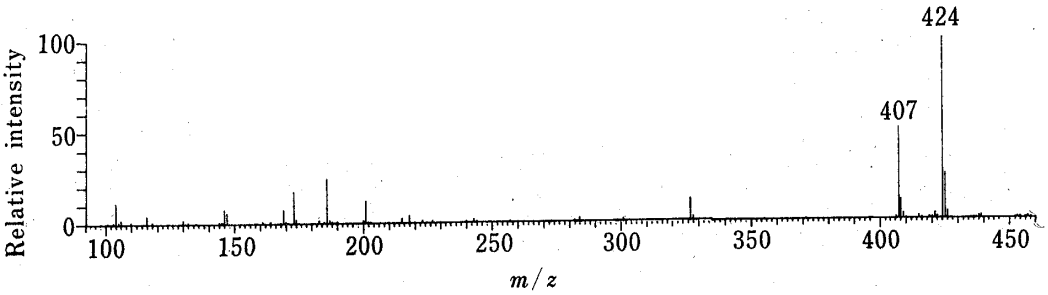

Fig. 4. CI–MS of the Dimethyl Ester of Palmitoyl–LPA

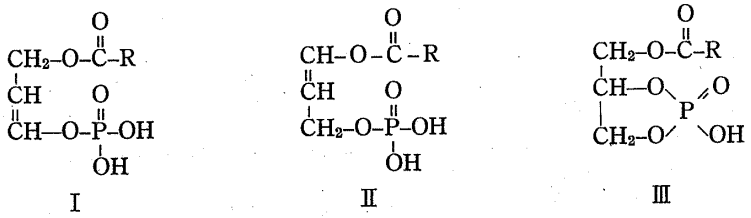

Chart 1

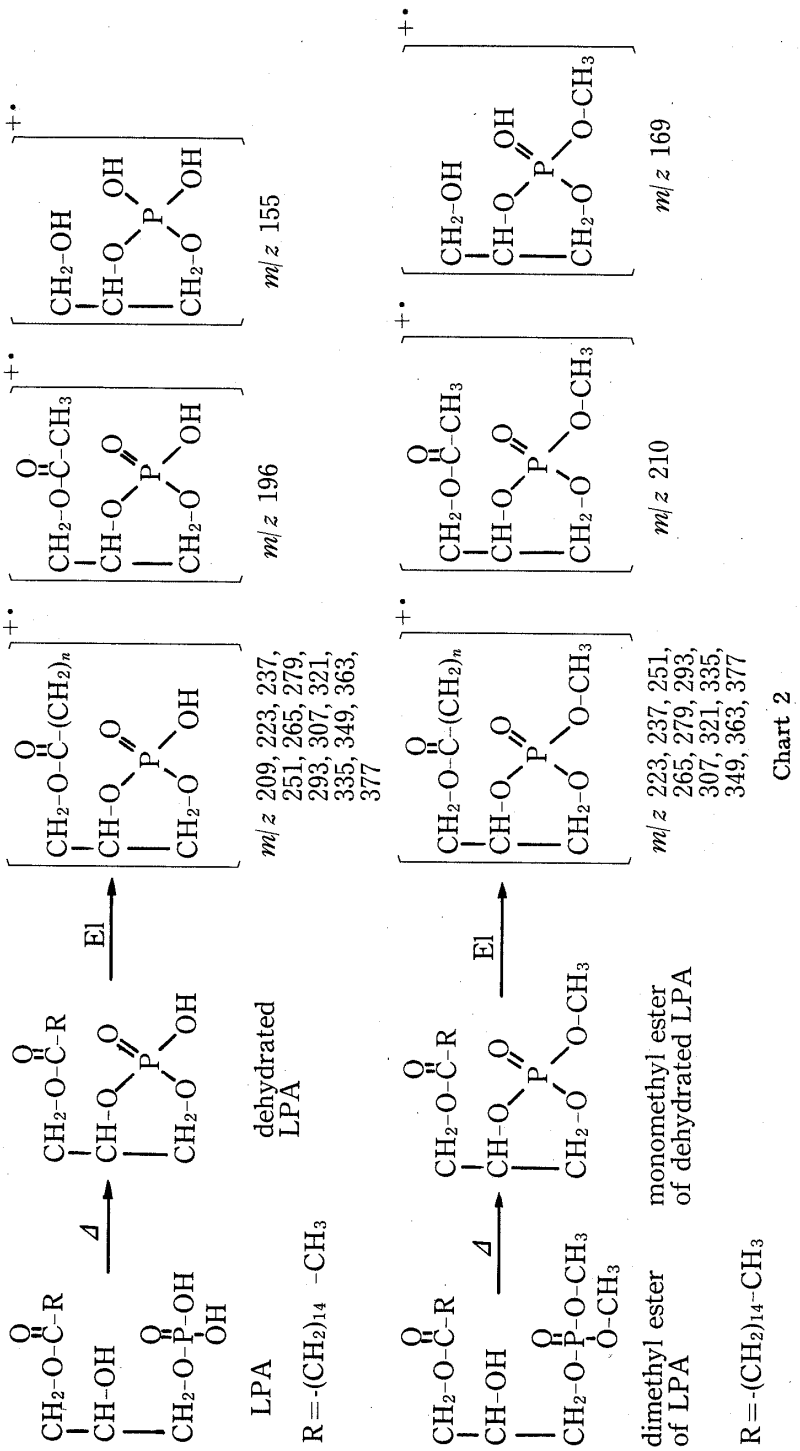

Chart 2

407 and *m/z* 424 (with ammonia) also shifted to *m/z* 410 and 427, respectively. These isotopic experiments supported the view that the dimethyl ester is converted to the monomethyl ester of dehydrated LPA on the heated probe and that a homologous series of phosphorus-containing fragment ions including base peak were formed by cleavage of the carbon-carbon bonds in the acyl moiety or deacylation by electron impact without essential structural changes in the phosphorus-associated part. The structures are suggested for major phosphorus-containing fragment ions (Chart 2).

1-Decanoyl-, lauroyl-, myristoyl- and stearoyl-LPA and their dimethyl esters showed structural changes on the heated probe similar to those of palmitoyl-LPA and its dimethyl

ester (elimination of H_2O or CH_3OH). In the EI–MS of LPAs with decanoyl, lauroyl, myristoyl and stearoyl groups, the dehydrated ions were seen at *m/z* 308, 336, 364 and 420, respectively. The patterns of fragmentation of the dehydrated LPAs were similar, yielding several fragment ion peaks that depended on the acyl chain length of each LPA and some common ion peaks. The fragment ion at *m/z* 155 was a base peak in the mass spectra of all LPAs examined. The ions assigned to $[M-CH_3OH]^{+\cdot}$ were detected at *m/z* 322, 350, 378 and 434 in the EI–MS of dimethyl esters of decanoyl-, lauroyl-, myristoyl- and stearoyl-LPA, respectively. The patterns of fragmentation were similar among the dimethyl esters, and the fragment ion at *m/z* 169 was a base peak in the mass spectra of all dimethyl esters tested. In the CI–MS of various LPAs, the ions assigned to $[M-H_2O]\cdot H^+$ (with methane and isobutane) and to $[M-H_2O]\cdot H^+$ and $[M-H_2O]\cdot NH_4^+$ (with ammonia) were observed. Similarly, the ions assigned to $[M-CH_3OH]\cdot H^+$ (methane and isobutane) and $[M-CH_3OH]\cdot H^+$ and $[M-CH_3OH]\cdot NH_4^+$ (ammonia) were predominant in the CI—MS of dimethyl esters of various LPAs.

In the analysis of LPAs, small amounts of another component were volatilized at 250—300°C and overlapped with the component assigned to dehydrated LPA, whereas a single component volatilized in the analysis of dimethyl esters of various LPAs. In addition to the fragment ion peaks observed in the MS of dehydrated LPA, several characteristic ion peaks are seen in the mixed spectra at intervals of 14 mass units over the mass range of 331—550. These ion peaks were found to have the general formula $C_nH_{2n-4}O_4$ by high resolution mass spectrometric analyses. The ion at *m/z* 550 appeared to be dehydrated dipalmitoyl-glycerol ion. At present, it is uncertain why diglyceride was produced during analysis of the monoacylphospholipid.

When the ionization energy was increased to 70 eV or the temperature of the ion source was increased to 300°C in the analysis of palmitoyl-LPA, the total ionization greatly increased, but the relative intensities of ion peaks with higher mass numbers were reduced. These changes in conditions did not influence the ratio of occurrence of heat-degraded products such as dehydrated dipalmitoyl-glycerol and dehydrated LPA. However, the amount of LPA applied to the probe influenced the ratio of formation of these components: when a larger amount of LPA was inserted, relatively more dehydrated dipalmitoyl-glycerol was detected by mass spectrometry.

Palmitoyl–LPA was dissolved in chloroform–methanol mixtures and aliquots were applied to the direct insertion probe. Total ionization increased progressively with rise of temperature

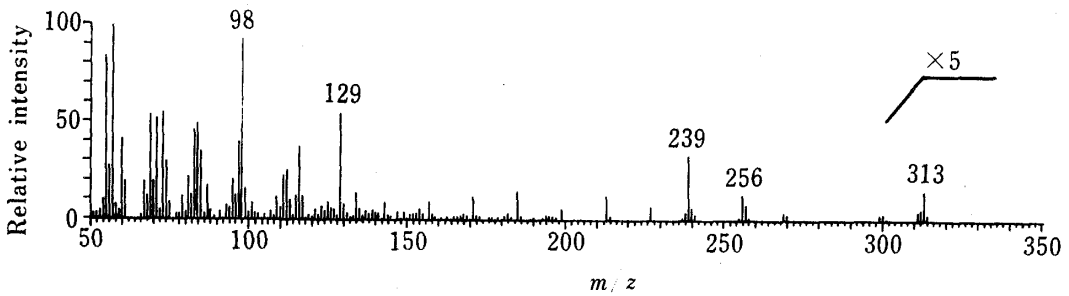

Fig. 5. EI–MS of Palmitoyl–LPA dissolved in Organic Solvents

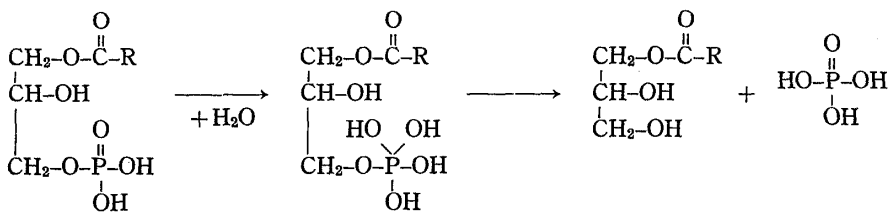

Chart 3

of the probe over 300°C. The EI–MS of the component is shown in Fig. 5. The base peak at *m/z* 98 was found to have a formula corresponding to $C_6H_{10}O$ (observed mass, 98.0747; calculated mass, 98.0732) by high resolution mass spectrometric measurements. This fragment ion is probably a cyclic ion formed by 6, 7 cleavage of the acyl moiety as suggested by Ryhage and Stenhagen.[18] Other characteristic fragment ions at *m/z* 129 ($[C_7H_{13}O_2]^{+\cdot}$), 239 ($[RCO]^{+\cdot}$), 256 ($[RCOOH]^{+\cdot}$) and 313 ($[M-H_2PO_4)^{+\cdot}$) had no phosphorus. These fragment ions in this spectrum were identical to those observed for palmitoyl-glycerol. Previous attempts[11–13] to analyze diacyl- and monoacyl-glycerophospholipids with or without silylation in the gas phase resulted in the detection of only the pyrolysis products, diacyl-glycerol and monoacyl-glycerol or their silylated derivatives, respectively, suggesting thermal cleavage of the phosphorus-oxygen bond in the phosphate ester as shown in Chart 3.

The present observations on LPA dissolved in organic solvents are consitent with those previously reported. Thus LPA itself is not volatilized, but thermally degraded to the dehydrated palmitoyl-glycerol at above 300°C under these conditions. Horning *et al.*[11,12] reported that portions of glycerophospholipids were converted into diacyl-glycerols in phenylether at 250°C and that addition of a small volume of water led to higher yields of diacyl-glycerols. Palmitoyl–LPA would be hydrated in organic solvents, and this hydration may prevent the cyclization between the glycerol moiety and the phosphate ester group, leading to dephosphorylation at higher temperatures. The dimethyl ester of palmitoyl-LPA, where two hydroxylgroups in the phosphate ester moiety are blocked, would not be hydrated. Therefore, it would be converted to the monomethyl ester of the cyclic form of palmitoyl-LPA, not to palmitoyl-glycerol, on the heated probe on insertion as either dry powder or solution.

The sensitivity of mass spectrometric analyses of LPAs and their dimethyl esters were determined. Certain amounts of LPAs dissolved in chloroform–methanol mixture (2: 1, v/v) were transferred into a quartz capillary tube. After drying *in vacuo* for 24 h, the tube was attached to the top of the probe which was then inserted into an ion source chamber and heated. Aliquots of chloroform solution of the dimethyl esters were put in a quartz capillary tube attached to the probe. Then the probe was inserted into an ion source chamber and heated. When LPAs and their dimethyl esters (more than 100 ng) were analyzed, satisfactory 20 eV-mass spectra were obtained for identification of these phospholipids. At higher ionizing voltage of 70 eV, 50 ng or more of LPAs and their dimethyl esters was necessary to characterize them. The ion $[M-H_2O]\cdot H^+$ and $[M-H_2O]\cdot NH_4^+$ were detected in CI–MS in amounts of more than 100 ng. The sensitivity of chemical ionization mass spectrometry of the dimethyl ester was equal to that of chemical ionization mass spectrometry of LPAs.

There are reports of successful analysis of intact polar phospholipids by electron impact and chemical ionization mass spectrometry.[14–16,19,20] These reports are much notable, however, the diagnostic phosphorus-containing ions were less abundant. In the present study, it was found that LPAs and their dimethyl esters were degraded on the probe in a specific manner, and that on electron impact these degraded phospholipids yielded a number of diagnostic phosphorus-containing fragment ions including a base peak. Chemical ionization gave much simpler spectra which are available for molecular weight determination of homologous series of LPA. The main disadvantage of this method is dephosphorylation of LPA into monoacyl-glycerols on their insertion into a probe in solution. The production of monoacyl-glycerol may be minimized by "various rapid heating-direct probe" methods and "close-probe" methods developed in the last few years.

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