Ammonia chemical ionization mass spectrometry of intact diacyl phosphatidylcholine

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Abstract Mass spectra have been obtained on molecular species of intact diacyl phosphatidylcholine by means of ammonia gas-induced chemical ionization. MH^+ ions were observed with all species, and other prominent ions in the spectra identified the fatty acid composition. Spectra of phosphatidylcholine containing deuterated methyl groups and spectra obtained using [^{15}N]ammonia have allowed identification of fragments containing choline methyl groups and ammonium adducts.—**Crawford, C. G., and R. D. Plattner.** Ammonia chemical ionization mass spectrometry of intact diacyl phosphatidylcholine. *J. Lipid Res.* 1983. **24:** 456–460.

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The determination of phospholipid structure and fatty acid composition by mass spectrometry has proved to be difficult due to their low volatility and tendency to decompose when heated. Although these problems have precluded routine mass spectral analyses of intact complex phospholipids, some success has been achieved. Klein (1, 2) has reported electron ionization (EI) mass spectrometric analysis of several intact phosphatidylcholine (PC) molecular species but could not detect a molecular ion. The analysis was further complicated by the anomolous fragmentation of the 1-stearoyl-2-oleoyl species. Field desorption (FD) mass spectrometry of intact phospholipids generally produces intense MH^+ ions (3), but interference by sodium or organic compounds requires that the samples be rigorously purified.

Foltz (4) reported that isobutane chemical ionization (CI) mass spectrometry of dioleoyl PC produced a spectrum that contained the MH^+ ion as well as fragments which defined the fatty acyl composition, but this promising technique has not been exploited. Here we report our results of CI mass spectrometric analysis of several intact diacyl PC molecular species and deuterated PC using [^{14}N] and [^{15}N] ammonia as the reagent gas.

MATERIALS AND METHODS

Dipalmitoyl, diheptadecanoyl, distearoyl, dioleoyl, 1-palmitoyl-2-oleoyl, and 1-oleoyl-2-palmitoyl PC were purchased from Sigma, Co., St. Louis, MO. Soy PC and phosphatidylethanolamine (PE) were purchased from Lipoid KG, Papenburg, West Germany. Dilinoleoyl and linoleoyl-linolenoyl PC were isolated from soy PC (5) and dilinolenoyl PC was prepared as described (6). PC containing deuterated methyl groups was prepared from soy PE (7) using CD_3I, purchased from Merck Co., St. Louis, MO, containing >99% deuterium. The deuterated PC was then separated into molecular species (5). The mass spectra were obtained on a Kratos MS-30 mass spectrometer equipped with a combined CI/EI source. CI spectra were produced at about 175 ev with a source pressure of approximately 1 torr. Samples (20–50 μg) in chloroform were put in glass capillaries and after solvent evaporation were introduced by direct insertion probe and heated to 250°C. The source temperature of the mass spectrometer was maintained at 250°C.

RESULTS AND DISCUSSION

The ammonia CI spectrum above m/z 200 of 1-palmitoyl-2-oleoyl PC shown in **Fig. 1** is typical of spectra obtained on all molecular species analyzed, and **Table 1** lists the ions observed for those species. The MH^+ ion was seen in the spectra of all molecular species, as was a more intense ion at MH^+-42. Proposed structures are shown in **Fig. 2.** For 1-palmitoyl-2-oleoyl PC the ion at m/z 577 (MH^+-183) contains both fatty acyl chains, and the ions at m/z 313 and 339 (R_1CO^+ + 74, R_2CO^+ + 74) contain one of the fatty acyl moieties that allows the identification of the molecular species. No substantial difference was seen in the spectrum of 1-oleoyl-2-palmitoyl PC, therefore R_1 and R_2 were not assigned

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; FD, field desorption; EI, electron ionization; CI, chemical ionization.

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

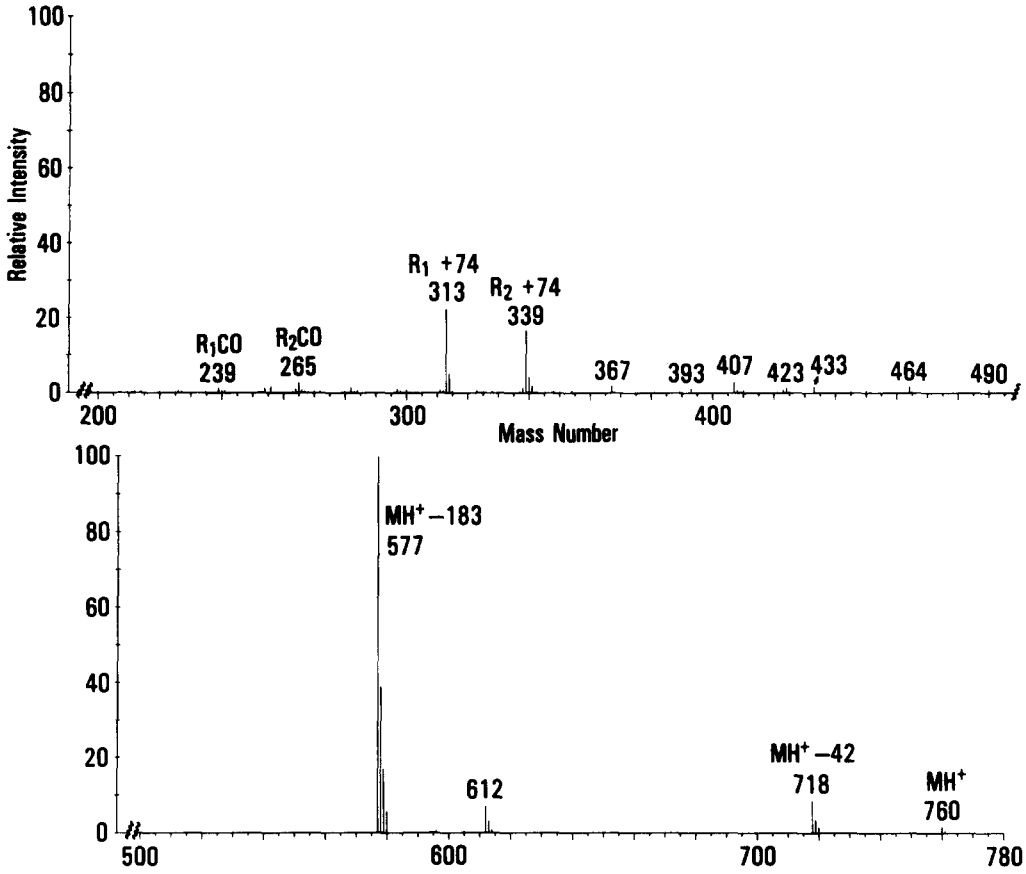

Fig. 1. Ammonia CI spectrum of 1-palmitoyl-2-oleoyl phosphatidylcholine.

on the basis of mass spectral analysis and are only used for descriptive purposes. The MH^+-183 ion was the most intense ion above m/z 200 in all CI spectra and did not show the hydrogen rearrangement reported to occur under EI conditions for some molecular species (1). Although FD spectra produce more intense MH^+ ions (3) than we have observed in CI, the minimal fragmentation in FD does not allow differentiation between some species (e.g., 1-stearoyl-2-linolyl and dioleoyl PC) readily seen in CI. Less intense ions at m/z 367 and 423 (R_1CO^+ + 128, R_1CO^+ + 184) and m/z 393 and 449 (R_2CO^+ + 128, R_2CO^+ + 184) are formed by CI as well as EI (1, 2). Other series of low intensity ions seen in this region are at m/z 407 and 464 (R_1CO^+ + 168, R_1CO^+ + 225) and m/z 433 and 490 (R_2CO^+ + 168, R_2CO^+ + 225), which may be analogous to the fragments found in EI spectra of trimethylsilyl derivatives of diacyl glycerol (8) and have the proposed structures shown in Fig. 2.

The most intense ions in all spectra were below m/z 200, with m/z 60 or 72 being the base peak and other major ions found at m/z 58, 90, 139, 142, 156, and

TABLE 1. Principal ions in ammonia CI mass spectra of phosphatidylcholine molecular species

	Molecular Species							
Fragment	16:0/16:0	16:0/18:1	17:0/17:0	18:0/18:0	18:1/18:1	18:2/18:2	18:2/18:3	18:3/18:3
MH^+	734	760	762	790	786	782	780	778
$MH^+ - 42$	692	718	720	748	744	740	738	736
$MH^+ - 183 + 35$	586	612	614	642	638	634	632	630
$MH^+ - 183$	551	577	579	607	603	599	597	595
$RCO^+ + 225$	464	464/490	478	492	490	488	488/486	486
$RCO^+ + 184$	423	423/449	437	451	449	447	447/445	445
$RCO^+ + 168$	407	407/433	421	435	433	431	431/429	429
$RCO^+ + 128$	367	367/393	381	395	393	391	391/389	389
$RCO^+ + 74$	313	313/339	327	341	339	337	337/335	335
RCO^+	239	239/265	253	267	265	263	263/261	261

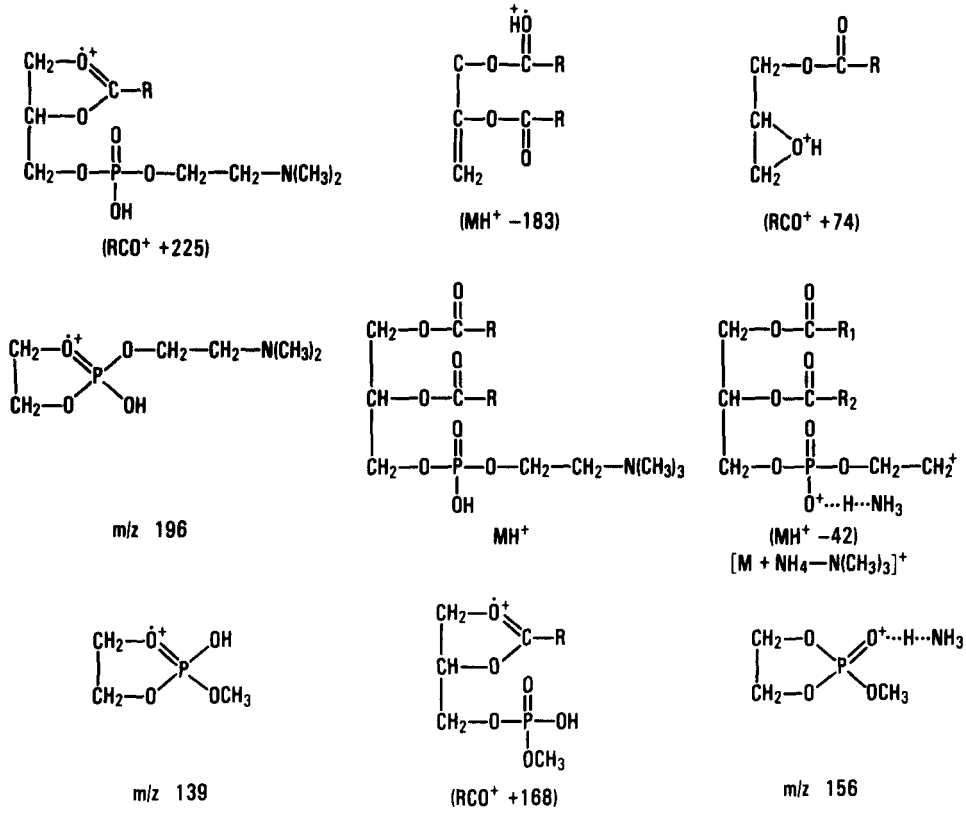

Fig. 2. Proposed structures of ions seen in spectra of intact diacyl phosphatidylcholine.

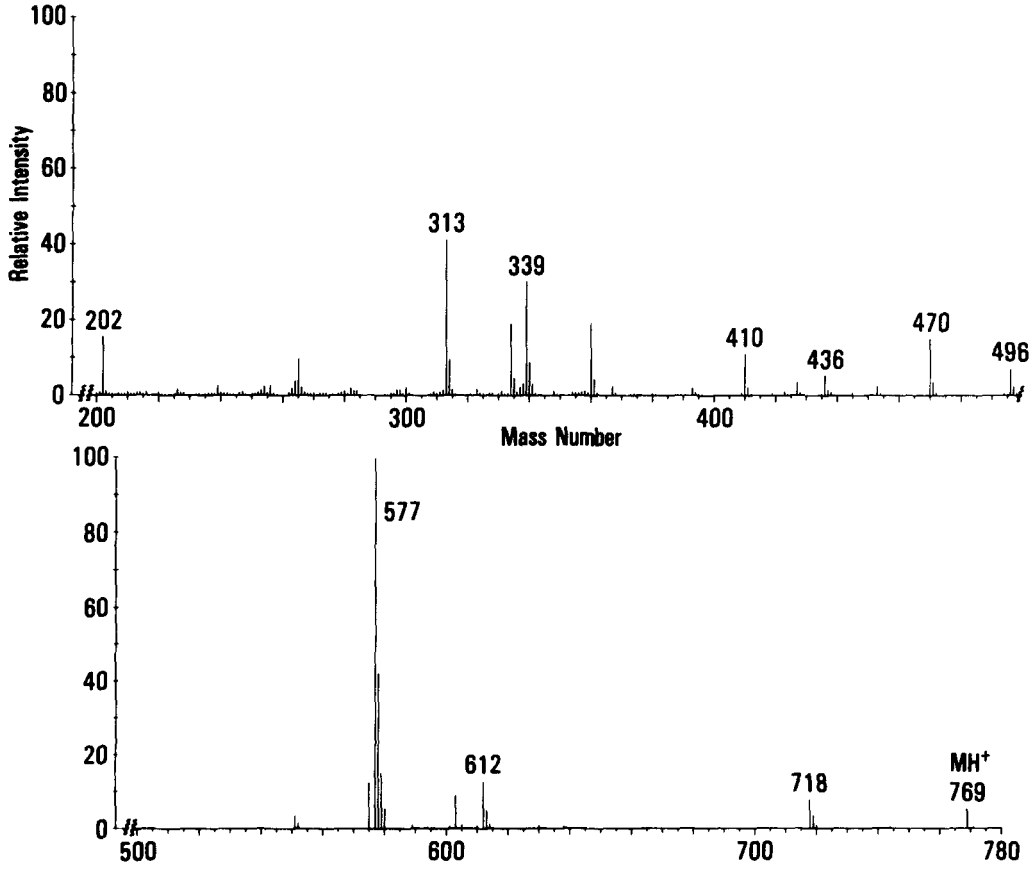

Fig. 3. Ammonia CI spectrum of 1-palmitoyl-2-oleoyl phosphatidylcholine containing deuterated methyl groups on choline.

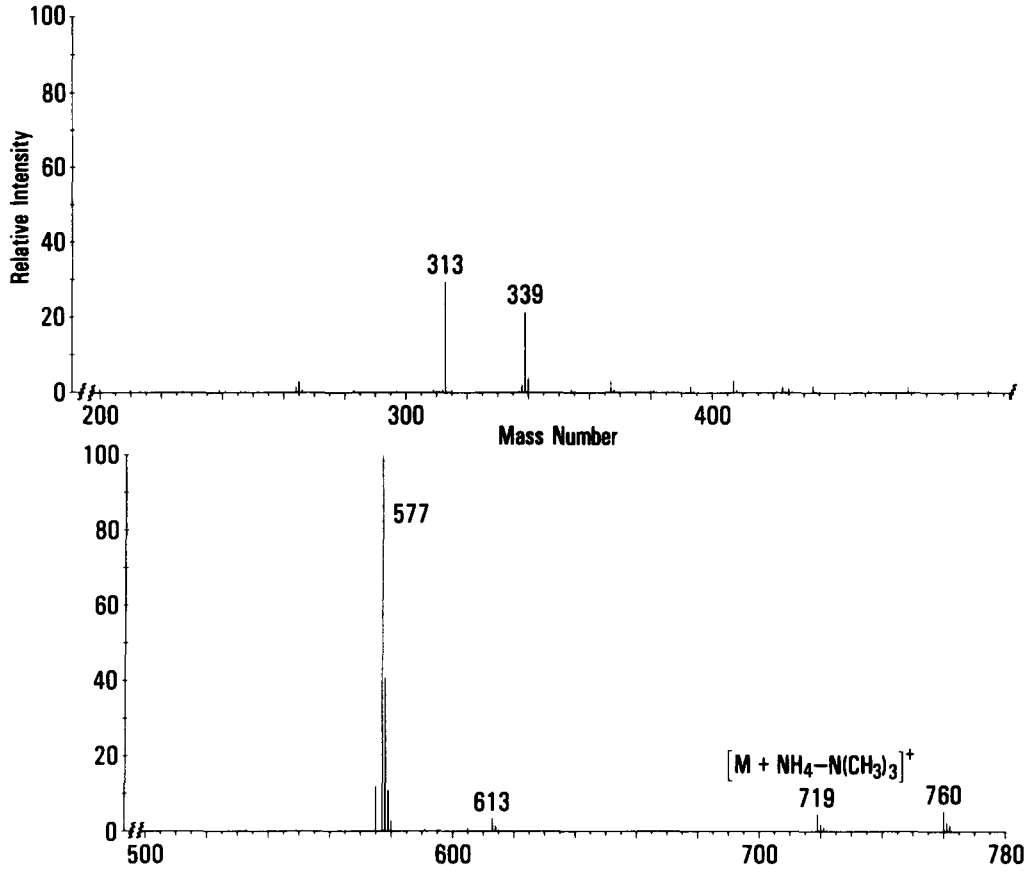

Fig. 4. [^{15}N]Ammonia CI spectrum of 1-palmitoyl-2-oleoyl phosphatidylcholine.

196. The fragment at m/z 60 was tentatively identified as $NH(CH_3)_3^+$, whereas those at m/z 72 and 90 have been identified previously (8, 9) as $CH_2{=}CH - N(CH_3)_2^+$ and $HO - CH_2 - CH_2 - NH(CH_3)_2^+$. Possible structures for ions at m/z 139, 156, and 196 are shown in Fig. 2. The origins of ions at m/z 58 and 142 are unknown, and their relative intensity varied between runs.

A sample containing >95% deuterated 1-palmitoyl-2-oleoyl was analyzed to elucidate fragments containing choline or methyl groups from choline, and the spectrum is shown in **Fig. 3.** The MH^+ ion increased by 9 amu as did m/z 60; whereas those at (RCO^+ + 225), m/z 196, 90, and 72 increased by 6 amu and those at (RCO^+ + 168) and 139 increased by 3 amu. Although the structures shown in Fig. 2 are merely proposed, the spectra of deuterated samples completely support the chemical formulae.

Unexpectedly there was no ion at MH^+-42 but there was an ion at MH^+-51, i.e., an ion at m/z 718 occurred in the spectrum of both deuterated and undeuterated 1-palmitoyl-2-oleoyl PC. A possible explanation is that trimethyl ammonium is lost and an ammonium adduct is formed. Support for this possibility was obtained using [^{15}N]ammonia as the reagent gas, and the resulting spectrum of undeuterated 1-palmitoyl-2-oleoyl PC is shown in **Fig. 4.** The ions at m/z 718, 612, and 156 seen in the spectrum of undeuterated 1-palmitoyl-2-oleoyl PC all increased by 1 amu, indicating that they are ammonium adducts, with the ion at m/z 718 resulting from the addition of ammonia and loss of trimethylammonium ion, that at m/z 612 being an ammonium adduct of diacylglycerol, and that at m/z 156 shown in Fig. 2.

These results show that CI mass spectrometry is a viable technique to identify molecular species of phosphatidylcholine but significant variation of ion intensities is seen in the spectra shown in the figures. Preliminary results acquired under carefully controlled instrument conditions show reproducible ion ratios that may allow quantitation of simple molecular species mixtures obtained after preliminary separation (5).

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