could be the result of a postulated[7c] hydrogen bond between the C-3 hydroxyl hydrogen and the Cer C-1′ carbonyl oxygen. This hydrogen bond would spread the two hydrocarbon chains, increasing association between adjacent molecules and thus increasing the stability of the model membrane system. The model presented in Figure 2 also illustrates this proposed interaction. On the basis of these data, Cer C-1 cannot be constrained to a ring as in the hydrogen-bonded network observed for crystalline cerebroside.[51] Rotations about both the Cer C-1—Cer C-2 bond and the Cer C-1—O bond would give Cer C-1 more mobility than the oligosaccharide group or Cer C-2 and C-3 separately and thus explain the larger value of NT_1 for Cer C-1. In this way, Cer C-1 would act as a double hinge between the oligosaccharide and ceramide groups.

There are two types of systems which have been used to study G_{D1a} in a membrane-like environment: micelles and vesicles (small unilamellar vesicles).[17] Micelles have a distinct advantage in this type of study since all resolvable resonances are observed without being obscured by resonances of the colipid necessary for ganglioside vesicles. Though a structurally simpler system than vesicles, there are several important similarities between the chosen experimental system and membrane bilayers. Micelles, like membranes, are spherical structures in which the head group is exposed to the aqueous medium. In both structures, the hydrophobic lipid chains are aggregated together away from the aqueous medium. Though there is no actual bilayer structure in micelles, each pair of micellar lipid chains is directly opposed by another pair of lipid chains as in a bilayer. These analogies show micelles to be a simple experimental model for membranes, and therefore the phenomena observed should represent properties of G_{D1a} in membranes to some extent. This is a particularly reasonable conclusion in the present case, since many of the properties observed for the very simple model systems such as sialic acid and the G_{M3} head group are also observed for G_{D1a} micelles.

Conclusion

With these studies, we have developed a qualitative understanding of the molecular dynamics and interactions of the major brain ganglioside, G_{D1a}, in a simple model membrane system. Significant among findings is the existence of a slight gradient in mobility among the sugar residues of the relatively immobile head group. Of particular interest in this regard are the distinct differences in conformational mobility observed for the structurally identical sialic acid residues. Groups attached to the saccharide rings were found to exhibit phenomena similar to neuraminyllactose,[2] and the first evidence for a previously postulated[7c] hydrogen bond in the ceramide backbone was acquired. These studies provide a comprehensive picture of gangliosides in a resting ground state, which is an essential basis for future experimentation involving these undoubtedly important glycolipids.

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Mass Spectrometry of Phospholipids.[1,2] Some Applications of Desorption Chemical Ionization and Fast Atom Bombardment

Eser Ayanoglu,[3a] Annemarie Wegmann,[3a] Olivier Pilet,[3a,b] G. Dean Marbury,[3c] J. Ronald Hass,[3c] and Carl Djerassi*[3a]

Contribution from the Department of Chemistry, Stanford University, Stanford, California 94305, and the Laboratory of Molecular Biophysics, N.I.E.H.S., Research Triangle Park, North Carolina 27709. *Received October 24, 1983*

Abstract: Major diagnostic peaks in desorption chemical ionization (D/CI) and fast atom bombardment (FAB) mass spectra of various model synthetic phospholipids and related compounds are reported in conjunction with our ongoing research on marine phospholipids. Similarities and differences with some previous studies are presented. Commercially available or partially synthesized, saturated or unsaturated fatty acid containing phospholipids with different head groups such as choline, ethanolamine, mono- and dimethylethanolamine, serine, and glycerol were investigated. For identification purposes, 1,2-diacetylglycerol, 1,2-diacetyl-*sn*-glycero-3-phosphocholine, and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 were also prepared and their mass spectral behavior studied. In terms of diagnostically useful fragmentations ammonia proved to be superior to methane as reagent gas for chemical ionization. The use of deuterated ammonia shed light on the nature of several fragmentations. In most cases, both chemical ionization and fast atom bombardment techniques exhibited molecular ions and/or related peaks. Desorption chemical ionization with ammonia provided more information about fatty acyl moieties, while fast atom bombardment gave diagnostic peaks about various head groups. These two techniques thus offer complementary information about the structures of intact phospholipids.

Phospholipids, together with sterols, are among the main classes of lipids in the cell membranes of living organisms. While electron-impact mass spectrometry has proved to be a very powerful technique in the structure elucidation of sterols, difficulties

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are encountered in providing useful spectra of phospholipids which display low volatility and thermal lability. Degradation and derivatization of such compounds from biological samples frequently interfere with a complete analysis of intact molecules. In order to overcome the above-mentioned problems a number of alternative ionization methods have been developed, and these have been the subject of several review articles.[4-6] Various classes of phospholipids have been examined by field-desorption mass spectrometry.[7-18] With the exception of phosphatidylserine, phospholipids provided intense MH^+ ions under these conditions. Further complications were encountered with phosphatidylcholines yielding "combination" or "association" ions due to the presence of the choline group, with many ions of higher mass than MH^+ having been observed.[5,9,10]

Chemical ionization[19-25] and fast atom bombardment[26,27,42] techniques were utilized for the analysis of several phospholipids but with two exceptions, no detailed and comparative study of the mass spectral behavior of intact molecules with different head groups and degrees of unsaturation has been conducted. One is a recent study[24] of the ammonia chemical ionization of diacylphosphatidycholine (vide infra) and the other[25] deals with the D/CI spectrometry of various phospholipids, but no experimental proof for the proposed fragmentations was presented.

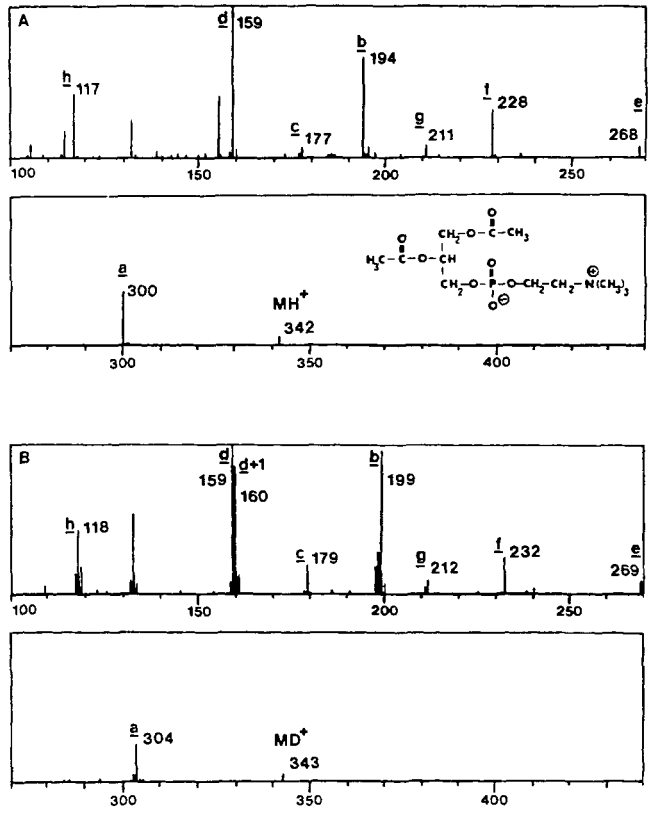

Figure 1. (A) D/CI mass spectrum of 1,2-diacetyl-*sn*-glycero-3-phosphocholine in NH_3. (B) D/CI mass spectrum of 1,2-diacetyl-*sn*-glycero-3-phosphocholine in ND_3.

Our recent research on the phospolipids of marine invertebrates[28-33] demonstrated that a wide variety of molecules containing different head groups and fatty acids is present in these organisms. As a consequence, we are now focusing on the chromatographic and spectral behavior of intact phospholipid species in order to investigate the possible role and biosynthesis of membrane components of marine organisms. As a first step we examined the mass spectral properties of commercially available or partially synthesized phospholipids with different head groups and fatty acids as model compounds. The latter included 1,2-diacetylglycerol and 1,2-diacetyl-*sn*-glycero-3-phosphocholine in order to eliminate any possible interference from fatty acid and/or polar head groups under the different ionization conditions. In addition, we used two labeled substances, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 and trideuterioammonia (ND_3) for confirming the course of some fragmentations. We now present our initial results using chemical ionization and fast atom bombardment techniques for the structure elucidation of phospholipids. The similarities and differences with earlier studies are also explored.

Experimental Section

1,2-Diacetylglycerol (**1**) was prepared by the addition of dry silver acetate to fresh 1,2-diiodohydrin in small portions at room temperature and crystalized from ether. Diiodohydrin was obtained from the reaction of allyl alcohol with excess powdered iodine at room temperature. The resulting crystals were washed with 0.01 N NaOH. 1,2-Diacetyl-*sn*-glycero-3-phosphocholine (**2**) was made by acetylation of L-α-glycero-

(1) (a) Part 263 in Mass Spectrometry in Structural and Stereochemical Problems. For Part 262 in this series see ref 31. (b) Part 6 in Phospholipids in Marine Organisms. For Part 5 in this series see ref 32.

(2) Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; DMPE, phosphatidyldimethylethanolamine; MMPE, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PA, phosphatidic acid; Shorthand notation of the fatty acids, number of carbon atoms, number of C=C double bonds (e.g., 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine, PC(16:0,18:2)); HPLC, high-performance column chromatography; TLC, thin-layer chromatography; MS, mass spectrometry; D/CI, desorption chemical ionization; FAB, fast atom bombardment; FD, field desorption.

(3) (a) Stanford University. (b) Present address: Dépertment Analytique, CIBA-GEIGY SA, 1870 Monthey, Switzerland. (c) National Institute for Environmental Health Sciences.

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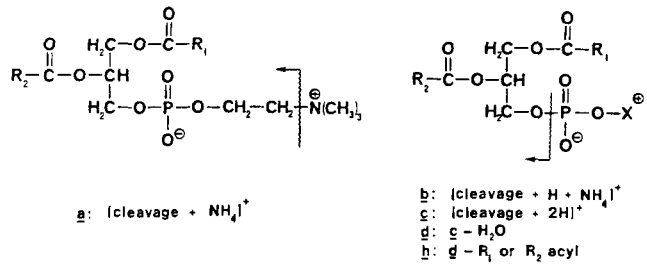

Figure 2. The formation of ions a, b, c, d, and h in the D/CI (NH_3) mass spectrometry of phospholipids. R_1 and R_2: fatty acyl chains. X: phospholipid head group.

phosphorylcholine cadmium chloride complex (Sigma Chemical Co.), following Khorana's procedure.[34]

Synthetic saturated and monounsaturated phosphatidylcholines (**3**, **4**, **5**, **7**, **13**), 1,2-dipalmitoyl-*sn*-glycero-3-phosphodimethylethanolamine (**8**), 1,2-dipalmitoyl-*sn*-glycero-3-phosphomonomethylethanolamine (**9**), and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (**10**) were purchased from Sigma Chemical Co. 1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (**14**) was made from 1,2-dipalmitoyl-*sn*-glycero-2-phosphocholine by treatment with phospholipase A_2[35] followed by acylation with linoleic acid. The acylation method was similar to that described by Khorana et al.,[34] but the anhydride was made in situ, using 1 equiv of dicyclohexylcarbodiimide (DCC).[36] 1-Palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoethanolamine (**15**) was prepared from **14** by enzymatic transphosphorylation with phospholipase D. The enzyme, extracted from Savoy cabbage,[37] was added in excess to the phosphatidylcholine dissolved in a 1:1 (v/v) mixture of ether and 4% aqueous ethanolamine hydrochloride (pH 5.7) containing $CaCl_2$ (0.04 M). The product was purified by HPLC on silica gel (E. Merck, 0.015–0.040 mm) with $CHCl_3$/MeOH (60:40 v/v) as eluent. Phosphatidylserines (**11**, **16**) and phosphatidylglycerols (**12**, **17**) were also prepared enzymatically according to literature methods.[38–40] 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 (**6**) was synthesized in quantitative yield from 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine with deuterated methyl iodide in $CHCl_3$/2 M K_2CO_3 1/1 (v/v) with benzyltrimethylammonium chloride as the catalyst.

Mass Spectrometry. (a) Desorption Chemical Ionization. All spectra were obtained on a Ribermag R-10-10 C quadrupole mass spectrometer, coupled with a PDP-8 data system using SIDAR software. The following CI source conditions were found to be optimum for our experimental purposes: the source pressure was kept at approximately 0.1 torr (source housing 2×10^{-4}) when NH_3 or ND_3 (99% isotopic purity obtained from Merck Sharp and Dohme Canada) was used as reagent gas. In general, the source temperature was kept at its lowest possible value (usually 100 °C). The D/CI coil with the sample was heated from 40 to 550 mA at 10 mA/s. Gold-plated tungsten coils were designed in our MS laboratory to minimize catalytic or thermal decomposition.

(b) Fast Atom Bombardment. The FAB spectra were acquired through the use of a VG-Analytical 70.70F mass spectrometer equipped with its standard FAB source. Instrument control and data acquisition functions were provided by means of a VG-Analytical 2050 data system. The mass scale calibrated with PFK with electron impact ionization and then checked for accuracy by examination of peaks of known mass in the FAB spectrum of glycerol. The samples were dissolved in glycerol for analysis. The use of dopants such as oxalic acid and NaCl did not improve the sensitivity of the technique. The current flow through the discharge was 1 mA with a beam energy of 7.0–7.5 keV. The ion-beam deflector was used to remove residual ions from the neutral beam. The spectra reported have been corrected for contaminating $(glycerol)_n + 1$ peaks by substraction of the FAB spectrum of glycerol obtained under the same experimental conditions.

Results and Discussion

Desorption Chemical Ionization (D/CI). The D/CI mass spectral data of phospholipids and related compounds with NH_3 and ND_3 are summarized in Table I. As a simple and illustrative example, the mass spectra of 1,2-diacetyl-*sn*-glycero-3-phosphocholine (**2**) in NH_3 and ND_3 are reproduced in Figure 1. The differences in the intensity of the corresponding ions for our compounds are partially due to the known fact that D/CI spectra are very dependent on constant source temperature (±5–10 °C) and source pressure. A 10-mA/s Au/W coil heating rate for the sample introduction provided better results than a slower 5 mA/s heating rate.

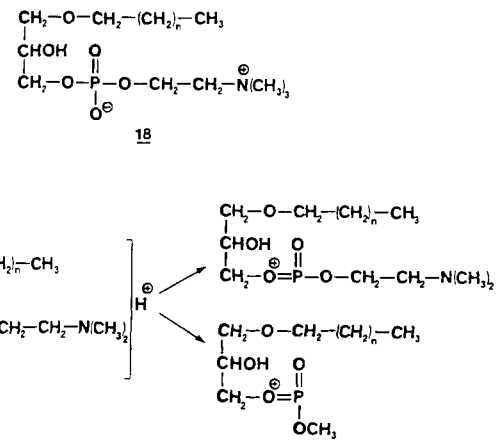

Figure 3. Proposed formation of rearrangement ions from **18**.[22]

With the exception of phosphatidylserines, all phospholipids exhibited protonated molecular ions. All phosphatidylcholines showed in addition an intense $MH^+ - 42$ ion (peak a, Table I, Figures 1A and 2). This fragment ion was not present in PE, PS, and PG species and suggested the involvement of the choline moiety which was mentioned in an earlier study from this laboratory,[23] namely cleavage of the trimethylamine group and formation of an ammonium adduct ($MH^+ - N(CH_3)_3 + NH_3$). In a recent study, such an involvement was also presented[24] as a possible explanation for the loss of 42 amu. Support for this possibility was obtained with [^{15}N] ammonia as the reagent gas which resulted in an $MH^+ - 41$ ion. In our studies, replacement of ammonia by ND_3 confirmed this explanation. The mass spectra of phosphatidylcholines, including **2** (Figure 1B), gave peaks of high intensity corresponding to the loss of 39 amu ions ($MD^+ - N(CH_3)_3 + ND_3$). For further proof, the mass spectra of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 were also recorded with both NH_3 and ND_3 as reagent gases. As expected NH_3 caused an intense $MH^+ - 51$ peak ($MH^+ - N(CD_3)_3 + NH_3$), while ND_3 provided a loss of 48 amu ($MD^+ - N(CD_3)_3 + ND_3$).

A diagnostically important fragmentation in the D/CI (NH_3) spectra of phospholipids is the fission between the phosphorus and the glycerol oxygen. Such a cleavage was first suggested[22] in a 1-*O*-alkyllysophosphatidylcholine (**18**, Figure 3), but no evidence was presented to confirm this suggestion. The mass spectra of the phospholipids investigated in our laboratory furnished several interesting peaks originating from this cleavage including the corresponding 1,2-diacyglycerol adduct, protonated, and elimination ions (Table I and Figure 1A, Figure 2, ions, b, c, and d).

Utilization of ND_3 as the reagent gas verified the formation of the ions b and c as well (Table I, Figure 1B). A +2 amu shift for the protonated ion c, and a +5 amu shift for the adduct ion b were observed. These shifts are attributed to the active hydrogen of the corresponding diacyglycerol, formed in the cleavage and protonation process. The presence of "active" hydrogen in chemical ionization mass spectrometry of alcohols, carboxylic acids, and other functional groups has been reported previously.[41] In the case of alcohols, protonated ions such as a molecular ion $[M + H]^+$ involves a deuterium exchange yielding the ion $[M - H + D + D]^+$ which causes a +2-amu shift in ND_3. Utilization of ND_3 as reagent gas also produces a +5-amu shift ($[M - H + D + ND_4]^+$, instead of $[M + NH_4]^+$ for the adduct ion, which

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Table I. Diagnostic Peaks from the Chemical Ionization Mass Spectra of Phospholipids and Related Compounds[a,b]

no.	compounds[c]	reagent gas	P^+	a	b	c	d	e		f		g		h	
								e_1	e_2	f_1	f_2	g_1	g_2	h_1	h_2
1	diacetin	NH_3	177		194	177	159							117	
			(36)		(100)	(36)	(44)							(38)	
		ND_3	179		199	179	159							118	
			(26)		(100)	(26)	(40)							(16)	
2	PC(2:0,2:0)	NH_3	342	300	194	177	159	268		228		211		117	
			(5)	(33)	(67)	(6)	(100)	(5)		(38)		(12)		(38)	
		ND_3	343	304	199	179	159	269		232		212		118	
			(3)	(28)	(97)	(22)	(100)	(9)		(25)		(12)		(44)	
3	PC(6:0,6:0)	NH_3	454	412	306	289	271	324		284		267		173	
			(21)	(100)	(54)	(15)	(88)	(5)		(8)		(6)		(17)	
		ND_3	455	416	311	291	271	325		288		268		174	
			(23)	(100)	(25)	(4)	(96)	(6)		(4)		(4)		(13)	
4	PC(14:0,14:0)	NH_3	678	636	530	513	495	436		396		379		285	
			(10)	(32)	(9)	(0)	(100)	(8)		(25)		(18)		(36)	
		ND_3	679	640	535	515	495	437		400		380		286	
			(5)	(14)	(3)	(0)	(100)	(12)		(5)		(21)		(51)	
5	PC(16:0,16:0)	NH_3	734	692	586	569	551	464		424		407		313	
			(26)	(100)	(16)	(4)	(96)	(10)		(14)		(8)		(18)	
		ND_3	735	696	591	571	551	465		428		408		314	
			(23)	(100)	(23)	(0)	(98)	(58)		(50)		(50)		(63)	
6	PC-d_9(16:0,16:0)	NH_3	743	692	586	569	551	470		427		410		313	
			(2)	(3)	(2)	(4)	(96)	(23)		(6)		(46)		(100)	
		ND_3	744	694	591	571	551	471		431		411		314	
			(8)	(12)	(7)	(0)	(60)	(70)		(41)		(69)		(100)	
7	PC(18:0,18:0)	NH_3	790	748	642	625	607	492		452		435		341	
			(30)	(100)	(21)	(0)	(100)	(10)		(6)		(17)		(21)	
8	DMPE(16:0,16:0)	NH_3	720		586	569	551							313	
			(8)		(32)	(3)	(78)							(100)	
9	MMPE(16:0,16:0)	NH_3	706		586	569	551							313	
			(19)		(26)	(0)	(100)							(37)	
10	PE(16:0,16:0)	NH_3	692		586	569	551							313	
			(33)		(100)	(4)	(91)							(17)	
11	PS(16:0,16:0)	NH_3	736		586	569	551							313	
			(0)		(0)	(0)	(2)							(35)	
12	PG(16:0,16:0)	NH_3	723		586	569	551							313	
			(15)		(82)	(0)	(100)							(60)	
13	PC(16:0,18:1)	NH_3	760	718	612	595	577	490	464	450	424	433	407	339	313
			(15)	(69)	(14)	(0)	(100)	(6)	(3)	(4)	(3)	(11)	(6)	(12)	(14)
14	PC(16:0,18:2)	NH_3	758	716	610	593	575	488	464	448	424	431	407	337	313
			(20)	(68)	(39)	(2)	(100)	(4)	(6)	(3)	(2)	(4)	(5)	(7)	(11)
15	PE(16:0,18:2)	NH_3	716		610	593	575							337	313
			(11)		(62)	(2)	(84)							(7)	(100)
16	PS(16:0,18:2)	NH_3	760		610	593	575							337	313
			(0)		(100)	(1)	(40)							(4)	(1)
17	PG(16:0,18:2)	NH_3	746		610	593	575							337	313
			(3)		(100)	(2)	(15)							(6)	(8)

[a] For abbreviations see ref 2. [b] See Figures 1, 2, and 4 and the text for the explanation of the ions. [c] Average intensities in parentheses.

accounts for the additional exchange of an "active" hydrogen for deuterium of the isotopic gas.

As complementary evidence for these fragmentations, one of our model compounds, 1,2-diacetylglycerol (diacetin) (**1**), which does not contain a phospholipid polar group or CH_2 chain, exhibited the same adduct (b, m/z 194), protonated (c, m/z 177), and elimination (d, m/z 159) ions as did 1,2-diacetyl-*sn*-glycero-3-phosphocholine (**2**). Replacement of NH_3 by ND_3 produced a +5-amu shift for the adduct (m/z 199) and a +2-amu shift for the protonated (m/z 179) ion in both compounds as expected. No difference was observed (cf. Table I) between the above-mentioned peaks of PC(16:0,16:0) (**5**) and PC-d_9(16:0,16:0) (**6**), indicating no involvement of the choline group. All these results confirm unambiguously a cleavage between the phosphorus and glycerol oxygen atoms and formation of related characteristic D/CI peaks. The ion at m/z 159 remained unchanged in the mass spectrum of diacetin when ammonia was replaced by ND_3. On the other hand, 1,2-diacetyl-*sn*-glycero-3-phosphocholine exhibited an additional d + 1 ion (Figure 1) when ND_3 was used, suggesting that d was partially formed by protonation of a neutral molecule in phospholipids. While the charged ion formed by the cleavage of the carbon–oxygen bond remains unchanged at m/z 159, the protonated one shifts +1 amu to m/z 160 due to the presence of ND_3. Ions b and d were mentioned in Crawford and Plattner's study[24] as $MH^+ - 183 + 35$ and $MH^+ - 183$, respectively, but the protonated diacylglycerol ion (ion c) was not reported and possible origins of d were not discussed. A hydrogen transfer occurs during the formation of c and other related ions. This hydrogen should come from the leaving head group moiety and cannot originate from the *N*-methyl group in the case of phosphatidylcholines, because no difference is observed between the corresponding peaks of PC(16:0,16:0) and PC-d_9(16:0,16:0) (Table I). Therefore one of the methylenes of the choline part of the molecule is the source of this transfer.

Another common and important diagnostic peak is the additional loss of one of the fatty acyl chains from the above-mentioned ion d. This fragmentation was observed in all cases, regardless of the changes in the fatty acyl or head groups (Table I, ion h). Phospholipids containing two different fatty acyl groups gave two corresponding peaks (h_1 and h_2).

In a previous study the presence of $MH^+ - 32$ and $MH^+ - 89$ ions was reported in the CI(NH_3) spectrum of a 1-*O*-alkyllysophosphatidylcholine.[22] It was proposed that these rearrangement ions arise from the loss of methanol and *N*,*N*-dimethylethanolamine, possibly by the transposition of one of the *N*-methyl groups to the phosphate group prior to fragmentation (Figure 3). We have observed similar ions, together with a loss of one of the fatty acyl groups (ions e and g). Since our substrates possess acyl groups

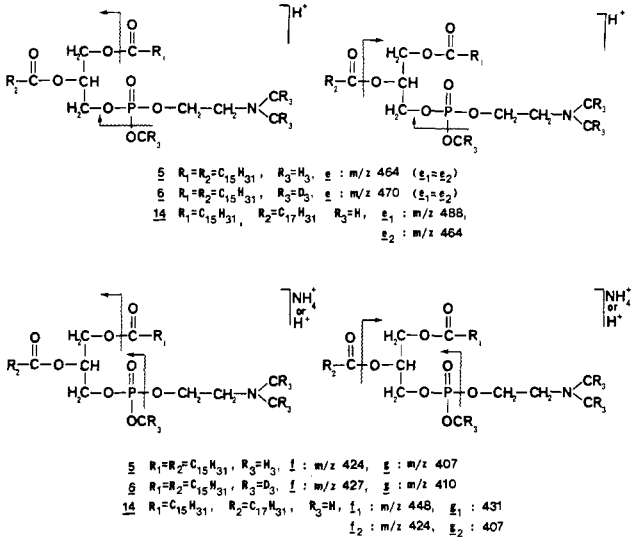

Figure 4. The formation of the rearrangement ions e, f, and g in the D/CI (NH_3) mass spectrometry of phosphatidylcholines. f: [cleavage + NH_4]$^+$. g: [cleavage + H]$^+$.

at both C-1 and C-2 of the glycerol backbone, rather than an ether bond at C-1 and a hydroxyl group at C-2 as in **18**, this additional loss is not surprising. For example, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (**5**) gave such an ion at m/z 464 (Figure 4). Dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 (**6**) exhibited it at m/z 470. The shift (+6 but not +9) clearly indicates the loss of one of the choline *N*-methyl groups. On the other hand, 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (**14**) showed two such fission ions at m/z 464 and 488 (e_1 and e_2), due to the presence of two different fatty acyl groups in the molecule.

The other rearrangement in (g), produced by the migration of one of the *N*-methyl groups to the phosphorus oxygen and loss of *N,N*-dimethylethanolamine together with one of the acyl groups, is also present in our phosphatidylcholines. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 (**6**) causes a +3 shift (one methyl group) in this ion (ion g) while it is not observed in our model diacetin (**1**), confirming the migration of one of the *N*-methyl groups (Figure 4). Phosphatidylcholines with two different fatty acids such as 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (**14**) again furnished two corresponding peaks (Table I, g_1 and g_2).

The ions e and g were also reported by Crawford and Plattner[24] as RCO$^+$ + 225 and RCO$^+$ + 128 (R = one fatty acyl group). The authors mentioned the presence of another ion at RCO$^+$ + 184, in the PC species they studied, arguing that they were formed by CI as well as EI. However, in our hands, CI produced ions at RCO$^+$ + 185, not at RCO$^+$ + 184, in each unlabeled phosphatidylcholine (Table I, ion f) including **2** (Figure 1). We did not detect it in PE, MMPE, DMPE, PG, or PS species. While 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine gave this ion at m/z 424, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 showed it at m/z 427, strongly suggesting that f is an ammonia adduct ion originating from g, which has only one migrated methyl group. As expected, use of ND_3 instead of NH_3 caused a +4-amu shift in each case, clearly demonstrating that f is in fact formed by the addition of reagent gas to g. Again phosphatidylcholine species with two different fatty acyl chains afforded two corresponding peaks (f_1 and f_2). In a more recent report,[25] the above-mentioned ion f was shown at m/z 424, not at m/z 423, in the ammonia chemical ionization mass spectrum of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine as another confirmation of our findings.

Replacement of methane as a reagent gas did not offer any advantages over ammonia, and in a number of instances it yielded more complicated results. Therefore these studies were not pursued further.

Fast Atom Bombardment. Fast atom bombardment under our conditions provided protonated ions in all cases with the exception of phosphatidylserines. This class of phospholipids is also known

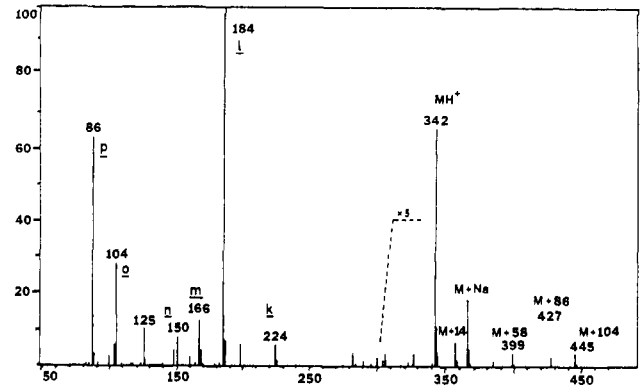

Figure 5. The FAB mass spectrum of 1,2-diacetyl-*sn*-glycero-3-phosphocholine (a small dimeric ion at m/z 683 is not shown in the figure).

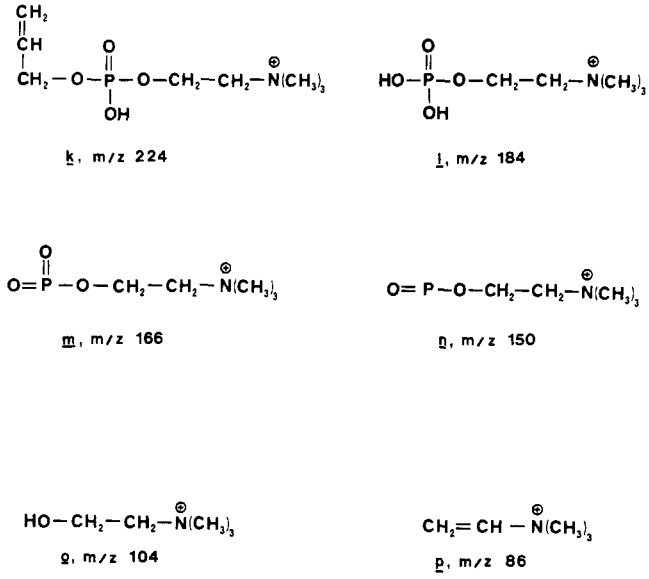

Figure 6. Polar-group fragmentation ions obtained from the fast atom bombardment mass spectrometry of phosphatidylcholines.

to fail in yielding useful field desorption spectra.[10] For comparison with desorption chemical ionization results and again as a simple and illustrative example, the FAB mass spectrum of 1,2-diacetyl-*sn*-glycero-3-phosphocholine is shown in Figure 5. 1,2-Diacetyl- and 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (**2** and **3**) also furnished dimeric ions of mass 683 and 908, respectively. Additionally, **2** showed small association ions at [M + 14], [M + 58], [M + 86], and [M + 104] together with an [M + Na] peak (Figure 5). These association ions were identified earlier in the field-desorption mass spectra of some phospholipids and were shown to originate from the intermolecular transfer of choline (mol wt 103) and its degradation products (loss of H_2O and loss of H_2O + $2CH_2$).[9,10] The acidic phospholipid phosphatidylglycerol gave [M + Na]$^+$ and [M + K]$^+$ peaks. Some common fragments reported earlier in the FAB and FD spectra of phospholipids[15,42] such as [H_3PO_4 + H]$^+$, [M - H_2PO_4]$^+$, [M + H - fatty acid]$^+$, and [M + H - fatty acyl chain]$^+$ were also encountered. Ions of small intensity, corresponding to the loss of one fatty acid and one fatty acyl chain, were also present in some cases.

Since these results were presented in the earlier studies, either in FAB of FD spectra of phospholipids,[9,10,15,42] further discussion will not be repeated in this report. However, the presence of diagnostic head-group peaks from various phospholipid classes using fast atom bombardment has not been extensively studied and offers an important advantage. The complicated nature of this mass range area in the D/CI mass spectra of phospholipids

(42) Aberth, W.; Straub, K. M.; Burlingame, A. L. *Anal. Chem.* **1982**, *54*, 2029–2034.

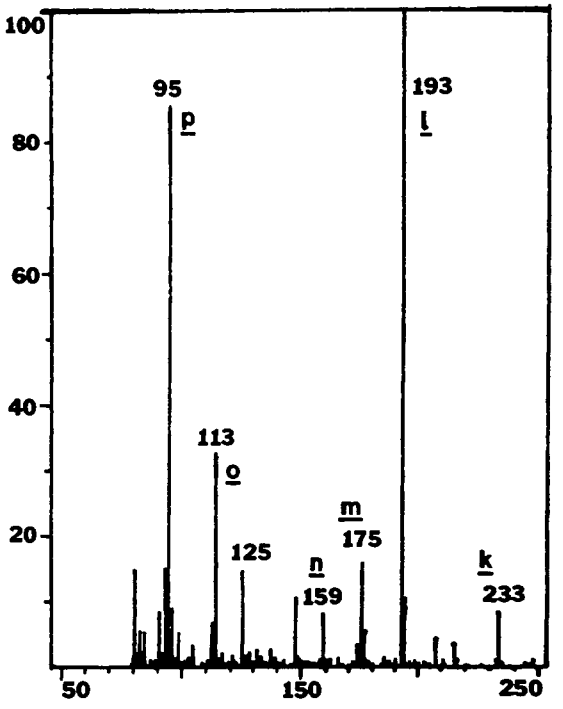

Figure 7. Diagnostic polar-group peaks in the FAB mass spectrum of dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9.

increases the importance of FAB results. Although the different phospholipid classes can be distinguished in most cases by TLC mobility and color reactions, in our hands, phosphatidylethanolamines and phosphatidylserines containing very long acids from marine organisms such as 5,9-hexacosenoic acid ($\Delta^{5,9}$ – 26:2) as their nonpolar moiety furnished higher R_f values than those of "conventional" ones, thus suggesting the misleading possibility of new head groups. On the other hand, lower R_f values were observed when new 2-OMe acids[30,31] were present, which again would lead to incorrect conclusions if one depended on TLC mobility.

All saturated and unsaturated phosphatidylcholines gave strong diagnostic peaks at m/z 86, 104, 150, 166, 184, and 224 (Figure 6) with the choline phosphate ion of mass 184 (**1**) always being the base peak. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine-d_9 (**6**) exhibited these peaks with +9-amu shifts (Figure 7) confirming the involvement of the choline moiety. The presence of choline (m/z 104) and dehydrocholine (m/z 86) is known[9] to occur in the mass spectra (FD) of phosphatidylcholines. The fragmentation ion of mass 166 (m, m/z 175 in PC-d_9(16:0,16:0)) is due to the loss of water from the choline phosphate ion **1** (m/z 184) as has already been noted in some alkane phosphonic acids.[43] The peak at m/z 125 is present in all PC's including **6** and can be depicted as the loss of the trimethylamine group from cholinephosphate.

(43) Griffithis, W. R.; Tabby, J. C. *Phosphorus* **1975**, *5*, 273–275.

Table II. Diagnostic Polar-Group Peaks from the Fast Atom Bombardment Mass Spectra of Amino-Containing Phospholipids[a,b]

phospholipid[c]	k	l	m	n
PC	224 (7)	184 (100)	166 (12e	150 (5)
PC-d_9	233 (8)	193 (100)	175 (14)	159 (6)
DMPE	210 (19)	170 (100)	152 (31)	136 (20)
MMPE	196 (33)	156 (100)	138 (43)	122 (11)
PE	182 (22)	142 (100)	124 (51)	108 (26)

[a] For abbreviations, see ref 2. [b] See Figure 5 and text for the explanation of the ions. [c] Average intensities, in parentheses.

In addition to phosphatidylcholines, phosphatidyldimethylethanolamines, phosphatidylmonomethylethanolamines, and phosphatidylethanolamines also gave peaks of diagnostic importance which still retain the polar groups. As can be noted from Table II, when compared with PC, they provide analogous peaks of 14 less atomic mass units, because of the decreasing number of *N*-methyl groups in these compounds. For example, instead of m/z 184 (**1**) in PC, base peaks were observed at m/z 170, 156, and 142 in DMPE (**8**), MMPE (**9**), and PE (**10**, **15**), respectively. Also, the FAB mass spectra of DMPE, MMPE, and PE furnished the above-mentioned head-group dehydration ions of respectively m/z 152, 138, and 124.

Fast atom bombardment mass spectra of phosphatidylglycerols (**12**, **17**) lacked the fragmentation peaks observed in PC, DMPE, MMPE, and PE. Instead, the expected glycerolphosphate protonated ion now appeared as the diagnostic polar-group peak (m/z 173), together with its sodium salt (m/z 195). Loss of water (m/z 155) from the phosphoglycerol ion was also observed. The most intense ion above m/z 100 was at m/z 115, corresponding to [glycerol + Na]$^+$.

In addition to these diagnostic ions at lower mass units, the two phosphatidylglycerols **12** and **17** studied by us provided intense peaks associated with the loss of glycerolphosphate ion (m/z 551 for PG(16:0,16:0) and m/z 575 for PG(16:0,18:2)). Subsequent loss of one of the fatty acyl groups was also observed (m/z 313 for PG(16:0,16:0) and m/z 337 as well as 313 for PG(16:0,18:2)).

In conclusion, the mass spectral behavior of phospholipids using desorption chemical ionization and fast atom bombardment techniques appears to provide valuable information about their molecular structures. Our results indicate that these techniques are complementary in the determination and structure elucidation of a variety of saturated and unsaturated phospholipids with different head groups.

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