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Direct Determination of Phospholipid Structures in Microorganisms by Fast Atom Bombardment Triple Quadrupole Mass Spectrometry

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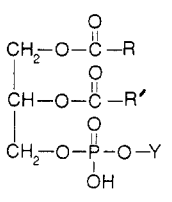
When phospholipids ionized by fast atom bombardment undergo collisionally induced dissociation (CID), they cleave at specific bonds between the functional groups contained on the lipid. These cleavages are common to all classes of phospholipids. By taking advantage of this fact, a general scheme has been developed that uses a triple-quadrupole mass spectrometer to rapidly characterize the phospholipid content and structures present in crude lipid extracts. This scheme is based on fast atom bombardment ionization of a crude lipid extract and on the combination of positive-ion neutral-loss and parent scans and negative-ion daughter scans. Neutral-loss and parent scans provide independent diagnostic mass spectra for each of many specific phospholipid classes, while daughter scans provide the emperical formulas and positions of the fatty acyl constituents on each phospholipid. An automated tandem mass spectrometry (MS/MS) instrument can perform an extensive phospholipid screening on a single sample. A useful mass profile of the phosphatidylethanolamine species present in a 1-pg sample of mixed phospholipids (equivalent to ten *Escherichia coli* cells) has been obtained. The spectra are reproducible and proportional to concentration over at least the five-logarithm range of cell concentrations studied. A rapid extraction procedure combined with the automated instrument control program produces profiles of the phospholipid classes, along with fatty acyl empirical formulas and position information, on selected phospholipid species, in a few minutes, from a single sample.

INTRODUCTION

The resurgence of interest in the mass spectrometry of phospholipids is due primarily to the development of fast atom bombardment (FAB) ionization and tandem mass spectrometry (MS/MS). Fast atom bombardment ionization allows the nonvolatile phospholipids to be analyzed without prior derivatization, and MS/MS allows phospholipid mixtures to be fully characterized without prior separation. Phospholipids are found in abundance in the cell membranes of living organisms. Phospholipid research has recently centered around the platelet activating factor (*1-3*), the use of phospholipids as biomarkers for bacteria (*4-6*) and algae (*7*), and the determination of general membrane function in living matter. Our interest in phospholipids stems from their usefulness as biomarkers for the detection and identification of microorganisms.

The phospholipids in which we are presently interested are the glycerophospholipids (structure I). Glycerophospholipids consist of four primary functional groups: a glycerol 3-phosphate core on which two fatty acids (R, R′) have been esterified to the two free hydroxyl groups in the *sn*-1 and *sn*-2 positions, and a second alcohol (Y) is esterified to the phosphate group in the *sn*-3 position (except for phosphatidic acid, which contains a protonated phosphate group). This head group (Y) is the functional group that defines the specific class to which the phospholipid belongs, while the fatty acyls distinguish the individual phospholipid molecular species within each class. Examples of different phospholipid classes are shown in Figure 1.



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For a method to be useful in obtaining a profile of microbial phospholipids in natural samples, it must have high sensitivity as well as high selectivity. While most of the phospholipid information is contained in the conventional FAB/MS spectrum (*4, 6*), the data are difficult to interpret; signals from lipid species at low concentrations are often buried in the high chemical background associated with FAB/MS, and FAB/MS does not work well for mixtures of phospholipids. Fast atom bombardment/tandem mass spectrometric analysis of daughter ions produced by collisionally induced dissociation (CID) of molecular ion precursors has proven useful for phospholipid structure analysis (*2, 8-13*). While this method is useful for obtaining information on the content, structures, and relative positions of the fatty acyls present on individual phospholipids, screening a mixture of phospholipids for the classes present would be a complex procedure requiring a mathematical analysis of the data set composed of the daughter spectrum from the ion current at every mass number. Constant neutral-loss scanning for polar head functional groups has been shown to be very useful for detection and differentiation of phospholipid classes in complex matrices and mixtures (*9, 14*).

All previous work on MS/MS analysis of phospholipids has been performed by using high-energy CID (*15*). The capability to perform these experiments on the triple-quadrupole mass spectrometer makes these analyses accessible to users of this type of instrument. In addition, the unit resolution for both mass analyzers becomes important when analyzing complex mixtures of phospholipids, such as microbial extracts, where lipid peaks might differ by no more than two mass numbers. Accomplishment of the analysis goals of speed and small sample size are facilitated by the control and automation available in the triple-quadrupole mass spectrometer.

When phospholipid ions undergo low-energy CID, only a few different fragment ion masses are formed. However, the fragment ions produced are the result of cleavages at specific points, which are common to all classes of phospholipids. These cleavages occur around the phospholipid functional groups and, thus, provide significant structural information about the parent ion. The low-energy CID fragmentation of phospholipids for both positive and negative ions is shown in Figure 2. In the positive-ion mode, the major reaction occurring is cleavage of the phosphate/glycerol bond, resulting in the loss of the polar head group as a neutral, while the rest of the ion retains the charge. The only exception we have found to this is the fragmentation of phosphatidylcholine. Phosphatidylcholine fragments so that the head group usually

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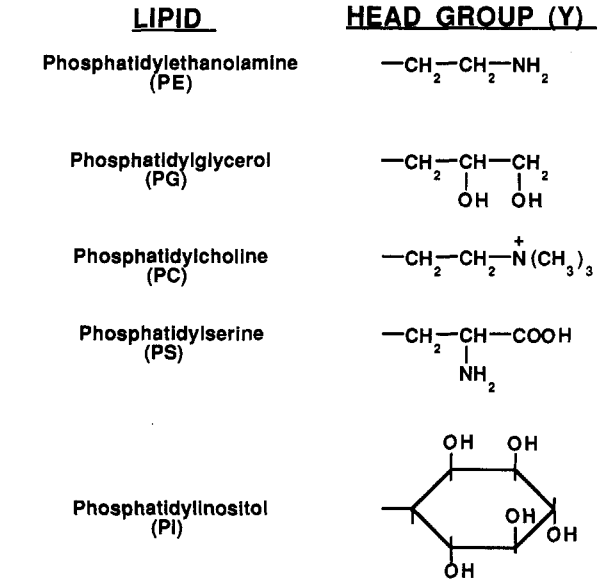

Figure 1. Examples of the head groups from five phospholipid classes.

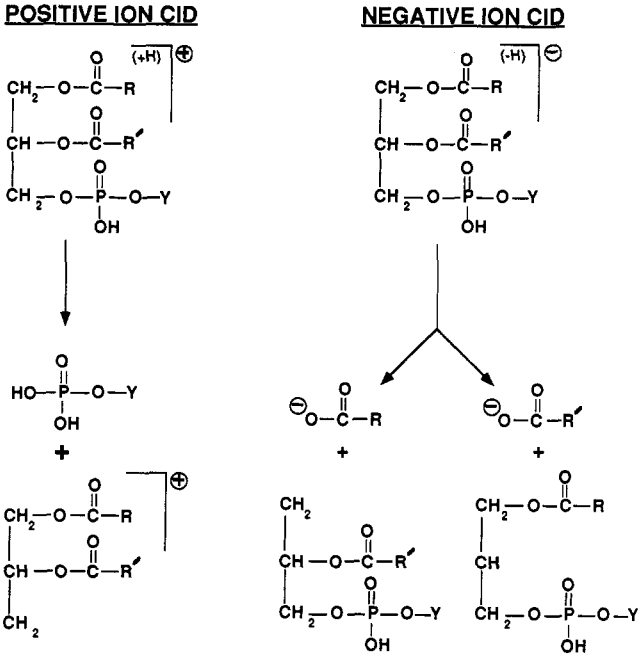

Figure 2. Low-energy CID fragmentation of phospholipids for positive and negative ions.

retains the charge or is lost as a sodium adduct, which causes difficulties in spectral interpretation. In the negative-ion mode, one or the other fatty acyl is cleaved and retains the charge while the rest of the ion is lost as a neutral. Examples of some of the possible positive-ion neutral losses and fatty acyl anions are given in Table I. A fragment cation of m/z 184 is used for the characterization of phosphatidylcholine. We have taken advantage of the fact that phospholipids always cleave around their functional groups to develop a general scheme for the rapid characterization of phospholipid classes present in microorganisms, including information on the fatty acyls present on the species within each class. This scheme is a sequence of positive-ion neutral-loss and parent scans and negative-ion daughter scans.

EXPERIMENTAL SECTION

All experiments were performed by using a Finnigan (San Jose, CA) TSQ-70B triple-stage-quadrupole instrument equipped with a standard Finnigan FAB source and a JEOL (Boston, MA) MS-009 charge-transfer FAB gun and power supply, which has been modified to fit and operate on the TSQ-70B instrument. Spectra were acquired and processed by using the Finnigan TSQ-70B data system and software.

Table I. Characteristic Positive-Ion Neutral Losses for Various Head Groups and Fatty Acyl Anions in the Daughter Ion Spectra

neutral losses,[a] u (group)	fatty acyl anions[b] (peak, m/z)	neutral losses,[a] u (group)	fatty acyl anions[b] (peak, m/z)
141 (PE)	C14:0 (228)	98 (PA)	C16:0 (255)
172 (PG)	C14:1 (226)	300 (LPG)	C16:1 (253)
185 (PS)	C15:0 (241)	155 (PMME)	C18:0 (283)
260 (PI)	C15:1 (239)	169 (PDME)	C18:1 (281)

[a] PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PMME = phosphatidylmethylethanolamine, PDME = phosphatidyldimethylethanolamine, PS = phosphatidylserine, PI = phosphatidylinositol, PA = phosphatidic acid, LPG = lysyl-phosphatidylglycerol. [b] C15:0 is a 15-carbon fatty acid with no unsaturation, C15:1 is a 15-carbon fatty acid with one point of unsaturation, etc.

All bacteria and fungi, except *Escherichia coli*, were obtained from researchers in the National Science Foundation's Center for Microbial Ecology at Michigan State University. Bacterial cultures were grown on trypticase soy agar plates at 37 °C for 24 h prior to harvesting. *E. coli* was obtained lypholyzed from Sigma Chemical Co. (St. Louis, MO).

Suspensions of *E. coli* were made by weighing out a known quantity of lypholyzed cells and suspending them in enough distilled/deionized water (DDI) to make a 1×10^9 cells/mL concentration using the conversion factor 5.9×10^{12} cells/g of dry weight (*16*). Dilutions of this suspension were made to obtain solutions of different cell concentrations.

Crude lipid extracts were obtained from the microorganisms by a modified Bligh–Dyer procedure (*17*). A 1-mL aliquot of a cell suspension or a transfer loop full of bacteria was placed in a clean, sterile 25-mL sample vial to which 15 mL of a 2:1 methanol: chloroform mixture was added. After the lipids were extracted, enough DDI water was added to separate the chloroform from the $MeOH/H_2O$. The chloroform layer, containing the lipids, was drawn off for analysis. For those experiments requiring a known cell concentration on the probe tip, the chloroform was blown off, by using a stream of nitrogen, until just enough solution remained to be placed on the probe tip for analysis (about 5 μL).

For the analysis, 3–5 mL of the chloroform solution was placed on the probe tip and dissolved in a drop of nitrobenzyl alcohol. Xenon was used as the FAB gas, and the FAB gun was operated with a filament current of 10 mA and a xenon beam energy of 8 keV.

Neutral-loss and parent ion spectra were obtained in the positive-ion mode with no CID gas added to the center quadrupole other than the residual gas remaining in the analyzer manifold. The fragmentation observed may be predominantly the result of metastable decomposition. Daughter spectra were obtained in the negative-ion mode by using argon as a collision gas. Collision gas pressure was 0.5 mTorr, and collision energy was 30 eV.

RESULTS AND DISCUSSIONS

Phospholipid data are obtained by this technique on two levels. The first level we refer to as the (*class*) *mass profiles*, which are the separate mass spectra for each phospholipid class. From these data, information is obtained regarding the phospholipid classes present, the masses of individual phospholipid species within each class, and the relative intensities of the species within each class. The second data level consists of the daughter spectra of individual phospholipid species and is referred to as (*class*)(*fatty acyl*) *formula data*. These data can be interpreted to provide empirical formula information on the fatty acyls and structural information on each phospholipid species. The data levels and their interrelation are

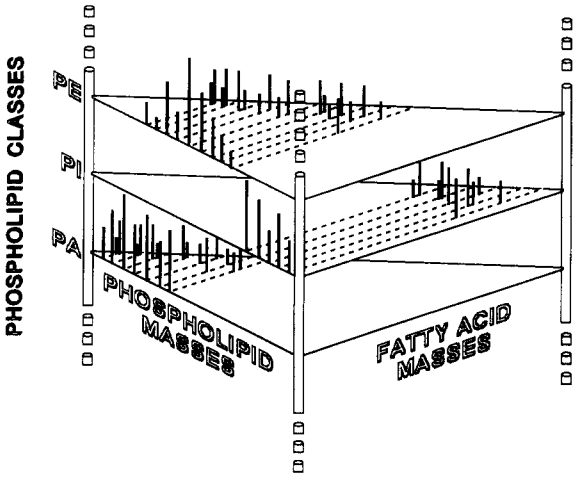

Figure 3. The FAB/MS/MS data space obtained by this technique. The masses of the phospholipids in each class are obtained by neutral loss or parent scans specific for each class. The daughter spectrum of each phospholipid species provides the masses and positions of the fatty acyls.

shown as a data space diagram in Figure 3. In this space, each phospholipid class forms its own data plane with the mass profile of its species contained along one dimension of the plane and the fatty acyl masses for each of its species along the other dimension. As discussed later, low-energy CID does not yet allow the complete structure of the fatty acyl groups to be obtained. The method described here collects (class)(mass) profiles and (class)(fatty acyl) formula data. Research is underway toward obtaining complete fatty acyl structural information.

General Profiling Scheme. The first step in the general scheme is to obtain the positive-ion neutral-loss and parent scans for the masses of the different phospholipid polar head groups. The instrument is programmed to scan a series of possible polar head group neutral losses and parent ions, thereby obtaining (class) mass profiles of all the phospholipid classes scanned. These profiles approximate the relative abundances of all species within each phospholipid class. The resulting spectra are free of any non-phospholipid peaks, and many peaks of interest that might have been hidden previously in the chemical background of the conventional mass spectrum show up clearly. An example of the use of neutral-loss scans for collecting (class) mass profiles of two of the phospholipid classes contained in *Proteus vulgaris* is shown in Figure 4. Note that the peaks due to phosphatidylglycerol (neutral loss 172 u) are completely buried in the chemical background of the conventional spectrum. This added detectability is a direct result of the additional selectivity afforded through tandem mass analyzers.

The relative intensities of the phospholipid species obtained in each (class) mass profile reflects the relative intensities of the species in the conventional mass spectrum. Apparent differences in intensities between the conventional spectrum and the neutral-loss and parent spectra are seen when more than one phospholipid class contributes to the abundance of a particular peak in the conventional mass spectrum. These overlaying peaks are resolved by the neutral-loss and parent spectra. Again, parent scans are used for the characterization of phosphatidylcholine. While the neutral-loss scan does work for phosphatidylcholine, we obtain greater sensitivity through the use of a parent scan for the head group ion. In addition to the increased sensitivity, these parent scans also discriminate against the appearance of peaks representing sodium adducts, thus providing cleaner spectra. A comparison of typical data obtained from neutral-loss and parent scans is shown in Table II.

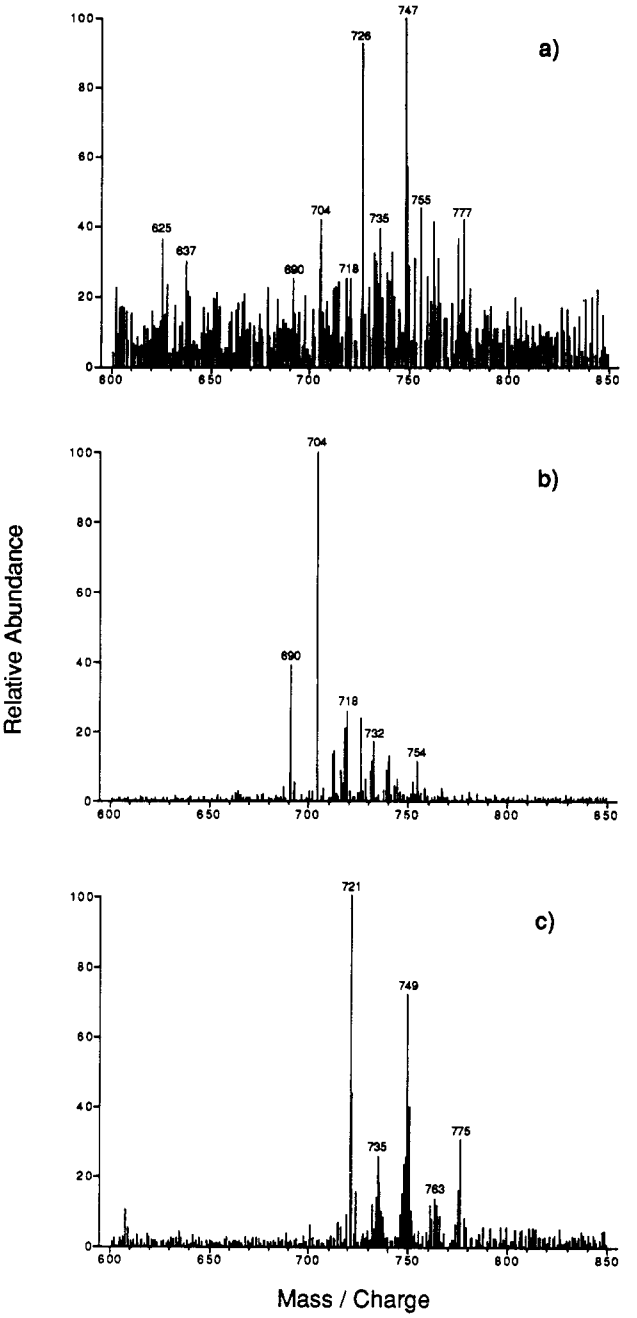

Figure 4. (a) Conventional mass spectrum of a *P. vulgaris* extract. (b) Neutral loss spectrum of 141 u; specific for PE. (c) Neutral loss spectrum of 172 u; specific for PG.

The two remaining pieces of information needed for a complete phospholipid profile are the composition, structure, and relative positions of the fatty acyls on the glycerophosphate core. Much of this information can be directly obtained from the negative-ion daughter spectra of the individual phospholipid species detected in the series of (class) mass profiles obtained from the neutral-loss and parent scans previously performed. As shown in Figure 2 for $[M - H]^-$ ion dissociation, the two major fragments present in a negative-ion daughter spectrum are the fragment ions due to the two fatty acyls contained on the phospholipid ion. The mass/charge value of the fragment ion corresponds to the molecular weight of the free fatty acyl, less one hydrogen, as shown in Table I. In this manner, the number of carbons contained in the fatty acyl may be determined, along with the degree of unsaturation. It is important to note that from these spectra, only the empirical formulas of the fatty acyls may be deter-

Table II. Comparison of Phosphatidylcholine (PC) Peak Intensities between Neutral-Loss and Parent Ion Scan Modes for Various Peaks Observed in Two Fungi Samples

PC peak, m/z	rel intensity, neutral-loss scan	rel intensity, parent ion scan
	Fungi 113-2	
754	ND[a]	3.4×10^5
757	ND	7.3×10^5
782	ND	7.3×10^6
784	ND	1.0×10^6
798	ND	1.7×10^5
754 + Na	2.4×10^5	ND
757 + Na	2.4×10^4	ND
782 + Na	6.2×10^4	ND
798 + Na	5.6×10^3	ND
	Fungi 101-6	
734	1.1×10^4	8.2×10^4
757	1.0×10^4	1.1×10^5
782	ND	2.5×10^5
798	ND	1.3×10^4
782 + Na	5.6×10^3	ND

[a] ND = not detected.

mined with certainty. At the present time, low-energy daughter spectra cannot determine the location of double bonds on a fatty acid, differentiate between a point of unsaturation and a cyclic (cyclopropyl) structure, or differentiate normal from branched fatty acids. However, others have shown that high-energy CID can be useful for solving some of these problems (*2*, *18*, *19*). Research using low-energy methods to solve these problems is presently underway in this laboratory.

The negative-ion daughter spectra also can provide information on the relative positions of the two fatty acyls on the phospholipid. Jensen, et al. (*2*) have postulated preferential formation of the carboxylate anion from the fatty acyl at the *sn*-2 position (closest to the head group) over that of the fatty acyl at the *sn*-1 position. When this is true, the daughter ion peak with the greater intensity is due to the fatty acyl from the *sn*-2 position. This work was performed by using high-energy CID. However, this same type of fragmentation has been observed with low-energy conditions (*12*), and we are applying this rule to our analysis. While this general rule apparently does not apply when phospholipids contain highly unsaturated fatty acyls or when large differences in the chain lengths of the two fatty acyls occur (*20*), it does hold for phospholipids containing fatty acyls with fewer than three points of unsaturation and with chain lengths differing less than 10 carbons from each other. Because fatty acyls that fit the exceptions to this rule are extremely rare in bacterial phospholipids, we are using this rule to obtain positional information about the fatty acyls present in our samples. The exceptional fatty acyls are easily diagnosed and the data treated accordingly. Figure 5 is an example of the use of negative daughter ions for *Salmonella abaetetuba*. The peak at m/z 733 represents the $[M - H]^-$ ion of a phosphatidylglycerol species. The two daughter ions obtained at m/z 255 and m/z 267 correspond to a C16:0 fatty acyl and a C17:cyc fatty acyl, respectively. As shown by the difference in peak intensities, the C17:cyc fatty acyl occurs in the first position of the phospholipid.

An automated instrument control procedure has been written that performs the necessary neutral loss or parent scans, in the positive-ion mode, for all nine phospholipid classes in which we are interested, switches the instrument into the negative daughter ion mode, and collects the individual daughter spectra from the 10 major peaks present in each of the phospholipid classes previously detected. The

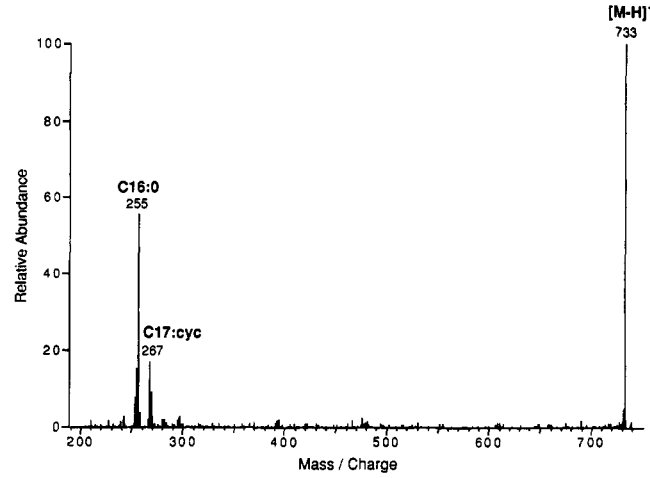

Figure 5. Negative-ion daughter spectrum of a phosphatidylglycerol species at m/z 733 showing the two fatty acyl ions and their relative intensities (C16:0 is a 16-carbon fatty acyl with no unsaturation; C17:cyc is a 17-carbon propyl fatty acyl). These assignments were made from correlating molecular weights with known fatty acid structures. From these correlations, m/z 255 most likely represents C16:0, and m/z 267 most likely represents C17:cyc or C17:1. Since the occurrence of C17:1 is somewhat rare in bacteria, m/z 267 most likely represents C17:cyc.

resulting data file contains up to the nine possible (class) mass profiles and the daughter spectra of up to 90 phospholipid species. The automated procedure can collect this information from a sample in under 10 min. A small portion of the complete data set used to obtain a phospholipid profile for *E. coli* is shown in Figure 6.

Sensitivity. The increase in selectivity due to the use of tandem mass spectrometry gives outstanding detection limits that are unattainable for these samples in conventional mass spectrometry. The top spectrum in Figure 7 is a neutral-loss scan for phosphatidylethanolamine (neutral loss of 141 u) of 1 pg of a mixture of phospholipids, which are mostly phosphatidylethanolamine species extracted from *E. coli*. This is roughly the phospholipid content of 10 *E. coli* cells. For comparison, the bottom spectrum shows the same scan of 1 μg of the same mixture; equivalent to 10^7 cells. All of the phosphatidylethanolamine species observed in the 1-μg sample have been detected in the 1-pg sample. This extremely low sample size requirement allows for the analysis of phospholipids at bacterial levels found in natural samples without prior cultivation of the organisms.

Precision. For this technique to be useful in bacterial phospholipid profiling, the spectra must be reproducible though varying concentrations of bacterial cells extracted. If the spectra changed with varying cell concentrations, the information regarding the classes, individual species, and fatty acid content of the phospholipids would be retained, but all information pertaining to the relative abundances of the individual species within each class would be dependent on the total quantity of phospholipid present. The degree of spectral variance obtained on our instrument for various cell concentrations is shown in Figure 8. By use of neutral-loss scans, the percent of the total phosphatidylethanolamine ion current was plotted as a function of the logarithm of the number of cells extracted for the major species present in *E. coli*. High precision was obtained, with the relative intensities of the masses within the spectra varying no more than 5% over at least a five-logarithm range of bacterial cell concentration.

Extraction Process. Related to the problem of spectral precision as a function of sample size is the problem of phospholipid recovery as a function of cells extracted. The recovery of this method was checked by analyzing cell extracts

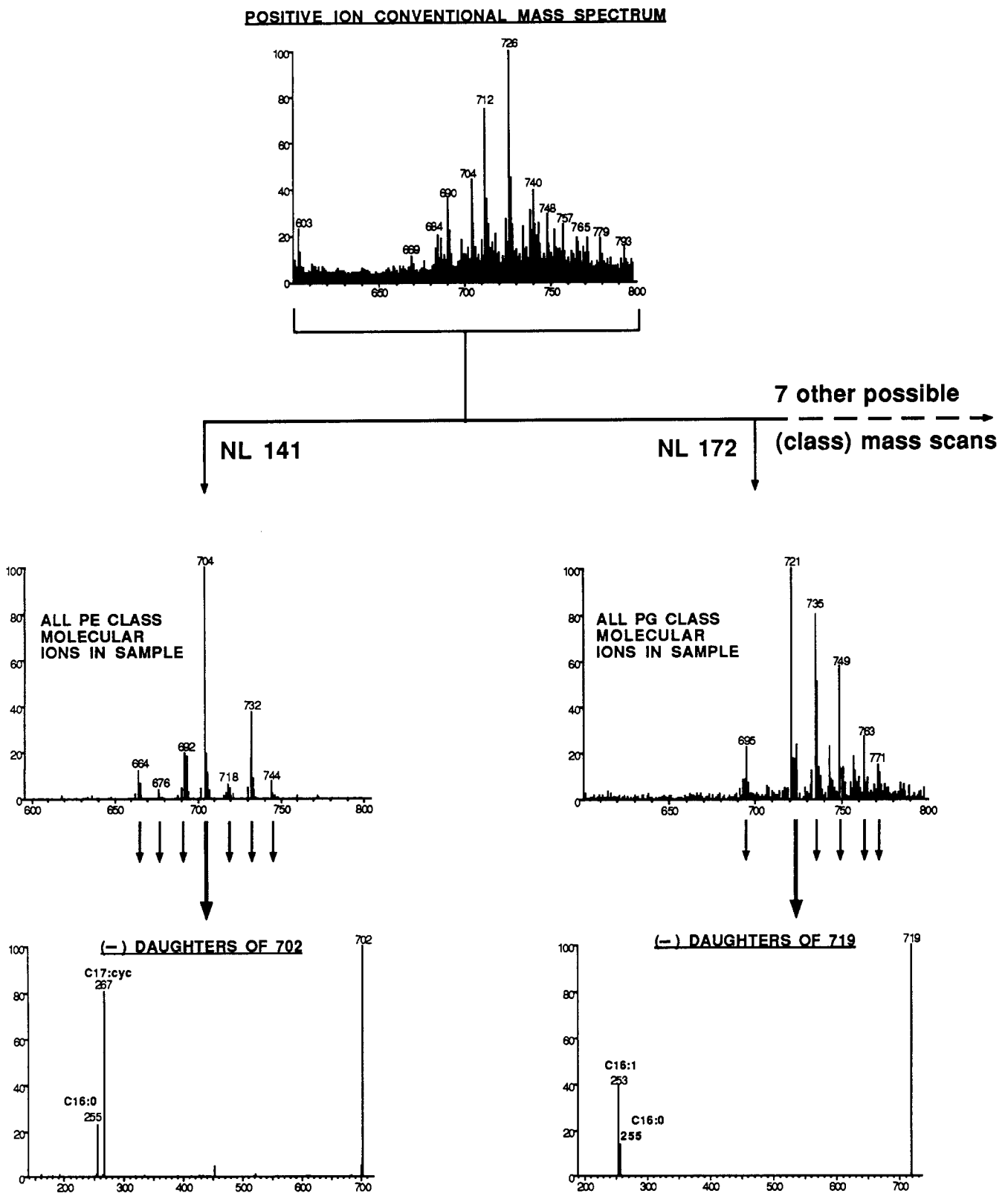

Figure 6. An example of the use of neutral loss and daughter spectra for obtaining a partial profile of *E. coli*. The neutral loss scans obtain the (*class*) *mass profiles*, while the daughter scans obtain the (*class*)(*fatty acyl*) *formula data*. As indicated, seven other neutral loss or parent scans are normally performed for a "complete" profile. Daughter spectra are obtained for the major peaks in each (*class*) *mass* scan.

of *E. coli* at various concentrations. The cells were extracted for 2 h before sampling. Neutral-loss scans were performed for phosphatidylethanolamine and phosphatidylglycerol, the two most abundant phospholipids in *E. coli*; the total ion current obtained was used as a measure of phospholipid abundance. Table III shows the logarithm of the total phospholipid ion intensity obtained for several values of the logarithm of the number of cells extracted. These data follow a linear fit with a correlation coefficient of 0.997, showing that the amount of phospholipid recovered in an extraction, relative to the number of cells extracted, remains constant.

With an automated instrument control procedure that can produce a phospholipid profile in under 10 min, the time required for extraction can become the limiting step with

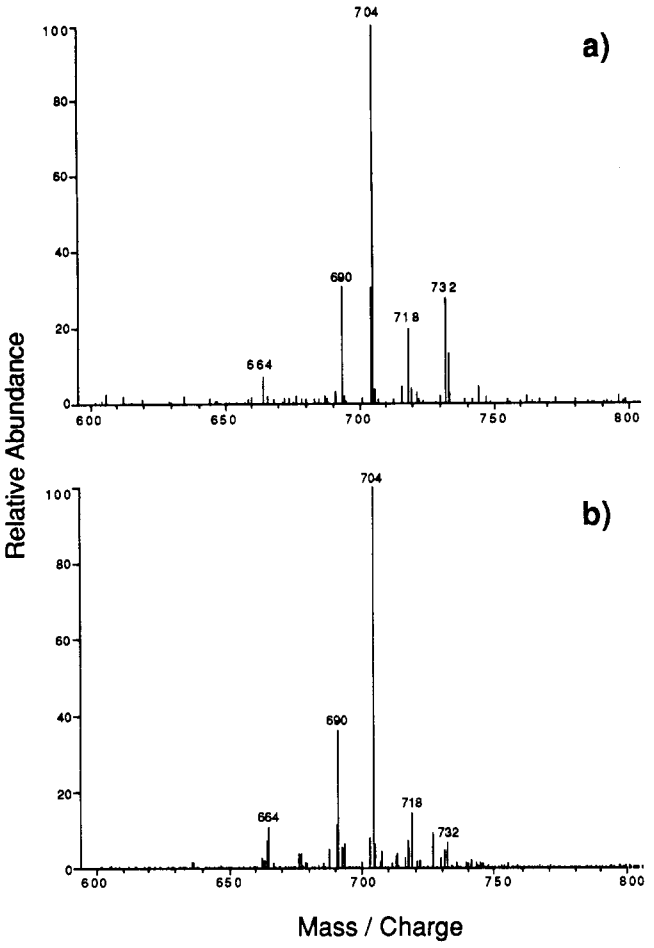

Figure 7. (a) PE mass profile of 1 pg of PE extracted from *E. coli*; equivalent to 10 cells. (b) PE mass profile of 1 μg of PE extracted from *E. coli*; equivalent to 10^7 cells.

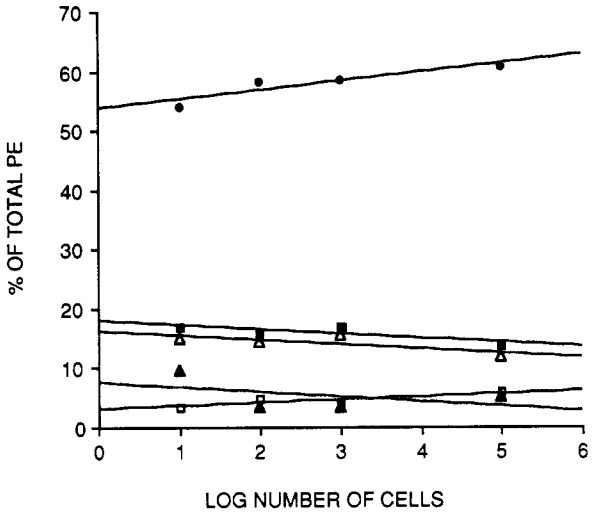

Figure 8. Percent of total PE as a function of the logarithm of the number of cells for the major species present in *E. coli*. Lipid ions: (□) *m/z* 664; (△) *m/z* 692; (●) *m/z* 704; (▲) *m/z* 718; (■) *m/z* 732.

Table III. Phospholipid Recovery for Various Values of log (Number of *E. coli* Cells Extracted)[a]

log (no. of cells)	log (lipid ion current)	log (no. of cells)	log (lipid ion current)
5.0	5.58	7.0	7.70
6.0	6.66	8.0	8.54

[a] Correlation coefficient = 0.997.

Table IV. Phospholipid Recovery from *E. coli* for Various Extraction Times

extraction time, min	no. of samples	rel ave phospholipid RIC,[a] × 10^6	rel std dev, %
1	3	3.87	23.4
10	3	4.54	23.3
60	3	4.49	28.2
120	3	4.77	28.9
1440	3	4.79	23.9

[a] RIC = reconstructed ion current.

regards to total analysis time. Typically, the samples are extracted for 24 h (*21*), with reports of more rapid extractions of 2 h (*22*). In an effort to decrease the total analysis time for a sample, the dependence of phospholipid recovery on the total extraction time was determined. Neutral-loss scans were performed on a series of three replicates each of five *E. coli* samples that had been extracted for 1 and 10 min and 1, 2, and 24 h, respectively. The total phospholipid ion intensities were used as a measure of the phospholipid recovery. Table IV shows the phospholipid recovery for various extraction times. These data suggest that little recovery advantage is gained after the first 10 min of extraction. In fact, a 1-min extraction yielded greater than 80% phospholipid recovery relative to the recovery obtained from the 24-h extraction. These rapid extractions significantly shorten the total analysis time and open up the possibility of performing on-line extractions coupled with a technique such as continuous-flow FAB.

CONCLUSIONS

The use of neutral-loss, parent, and daughter scans in combination with rapid extractions provides a quick and sensitive method for profiling the phospholipid content and distribution in microorganisms. Since bacteria maintain a characteristic and rather constant membrane phospholipid content, this method has great potential for detecting and identifying bacteria on the basis of phospholipid profiling. Research in this area is presently underway in our laboratory.

This technique is not restricted to use in bacterial phospholipid profiling; it can be used as a general method for any sample containing phospholipids. We are using this same method to characterize the phospholipid content of plants, fungi, and amoebae. However, the caveat about fatty acyl position data must be taken into account in working with samples that might contain fatty acyls included in the exceptions mentioned earlier. Many plant species contain highly unsaturated fatty acyls and/or large differences in the chain lengths of the two fatty acyls present on the phospholipids.

Finally, the basic concepts used in this approach also can be used to profile types of lipids other than glycerophospholipids. These concepts are being applied to the analysis of the content and distribution of glycolipids in certain bacteria and sterols in fungi. Applications to phosphonolipids, ether lipids, and other types of lipids are certainly possible.

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Separation and Identification of Organic Gunshot and Explosive Constituents by Micellar Electrokinetic Capillary Electrophoresis

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Micellar electrokinetic capillary electrophoresis (MECE) provides rapid and efficient separation and detection of organic gunshot and explosive constituents. Twenty-six of these constituents were separated in under 10 min with efficiencies in excess of 200 000 theoretical plates. The effects of the following experimental parameters were studied: sodium dodecyl sulfate (SDS) concentration, pH, addition of a tetraalkylammonium salt, capillary diameter, and injection times. The presence of gunshot residues in spent ammunition casings and the composition of six reloading powders and four plastic explosives were determined by using the MECE method. Multiple-wavelength analysis provided UV spectral profiles of the constituents for use with selective wavelength monitoring.

INTRODUCTION

Forensic investigators use the analysis of gunshot and explosive residues to identify materials and individuals involved in a crime. Various methods, including atomic absorption and neutron activation analysis, have been used to analyze the inorganic constituents of gunshot primer residues. Routine application has been limited because of interferences, high blanks, prohibitive instrument cost, and analysis time. Since commercial ammunition and explosives contain mixtures of explosives, stabilizers, and plasticizers, analysis of these characteristic organic constituents, listed in Table I, has gained recent attention. Some work has been done to analyze the organic constituents of gunshot residues by HPLC (*1*), GC/MS (*2*), and SFE/SFC (*3*), but none has gained widespread utility and application, as indicated by a recent survey of forensic laboratories (*4*). The thermally stable organic constituents of explosive materials are commonly analyzed by GC/MS (*5*), whereas LC-EC (*6*), LC-TEA (*7*), and SFC/UV (*8*) are used for the more thermally labile constituents.

Separations by capillary electrophoresis (CE) provide a number of advantages (*9*); of particular interest for forensic

Table I. Compounds Studied

Gunpowder Constituents	
dibutyl phthalate	DBP
N,N'-diethyl-*N,N'*-diphenylurea (ethylcentralite)	EC
2,3-dinitrotoluene	2,3-DNT
2,4-dinitrotoluene	2,4-DNT
2,6-dinitrotoluene	2,6-DNT
3,4-dinitrotoluene	3,4-DNT
diphenylamine	DPA
1,2,3-propanetriol trinitrate (nitroglycerine)	NG
nitroguanidine	NGU
2-nitrodiphenylamine	2-nDPA
N-nitrosodiphenylamine	N-nDPA
High-Explosive Constituents	
dibutyl phthalate	DBP
diethylene glycol dinitrate	DEGDN
1,3-dinitronaphthalene	1,3-DNN
1,5-dinitronaphthalene	1,5-DNN
1,8-dinitronaphthalene	1,8-DNN
ethylene glycol dinitrate	EGDN
1,2,3-propanetriol trinitrate (nitroglycerin)	NG
nitroguanidine	NGU
2-nitronaphthalene	2-MNN
2-nitrotoluene	2-NT
3-nitrotoluene	3-NT
4-nitrotoluene	4-NT
pentaerythritol tetranitrate	PETN
picric acid	PA
2,4,6,*N*-tetranitro-*N*-methylaniline	Tetryl
1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane	HMX
2,4,6-trinitrotoluene	TNT
1,3,5-trinitro-1,3,5-triazacyclohexane	RDX

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