MALDI-TOF/MS analysis of archaeabacterial lipids in lyophilized membranes dry-mixed with 9-aminoacridine

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Abstract A method of direct lipid analysis by MALDI mass spectrometry in intact membranes, without prior extraction/separation steps, is described. The purple membrane isolated from the extremely halophilic archaean Halobacterium salinarum was selected as model membrane. Lyophilized purple membrane were grinded with 9-aminoacridine (9-AA) as dry matrix, and the powder mixture was crushed in a mechanical die press to form a thin pellet. Small pieces of the pellet were then attached to the MALDI target and directly analyzed. In parallel, individual archaeabacterial phospholipids and glycolipids, together with the total lipid extract of the purple membrane, were analyzed by MALDI-TOF/MS using 9-AA as the matrix in solution. Results show that 9-AA represents a suitable matrix for the conventional MALDI-TOF/MS analysis of lipid extracts from archaean microorganisms, as well as for fast and reliable direct dry lipid analysis of lyophilized archaeabacterial membranes. This method might be of general application, offering the advantage of quickly gaining information about lipid components without disrupting or altering the membrane matrix.—Angelini, R., F. Babudri, S. Lobasso, and A. Corcelli. MALDI-TOF/MS analysis of archaeabacterial lipids in lyophilized membranes dry-mixed with 9-aminoacridine. J. Lipid Res. 2010. 51: 2818–2825.

Supplementary key words glycerolipids (diphytanylglycerol ether analog) • glycerophospholipids (diphytanylglycerolphosphate ether analog) • Halobacterium salinarum • purple membrane • matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Today shotgun ESI-MS lipidomics represents one of the most powerful tools of analysis of the total lipid extract of biomembranes, allowing the detection of both zwitterionic and acidic phospholipids (1).

Recently, a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) approach, based on the use of 9-aminoacridine (9-AA) as matrix, has been developed for the rapid analysis of glycerolipids, allowing a rapid, sensitive, and highly informative approach for the direct analysis of cell lipid extracts (2). Notably the use of the novel matrix 9-aminoacridine in MALDI-TOF/MS enables the fast and reliable detection of anionic phospholipids and also cardiolipin species (2).

In our study we extended the use of 9-aminoacridine as a matrix for the MALDI-TOF/MS to glycerolipids and glycerophospholipids of extremely halophilic microorganisms belonging to the Archaea kingdom. The extremely halophilic archaean are well adapted to life in NaCl-saturated aquatic ecosystems, where they are exposed to extremely low water activity and desiccation stress.

Glycerolipids and glycerophospholipids are defined as glycerol and glycerol-phosphate derivatives, respectively (3). Whereas in bacteria and eukaryotic cells, glycerolipids and glycerophospholipids are constituted by straight-chain fatty acids linked to glycerol by ester bonds, in the archaean microorganisms, the hydrophobic chains are isopropyl alcohol linked to glycerol by ether bonds (4).

The stereochrome of archaean biomembranes lipids is also different from that of other membrane lipids. In archaeabacterial lipids, the glycerol group is alkylated at the sn-2 and sn-3 positions, at variance from bacterial and eukaryotic lipids, whose chains are linked at the sn-1 and sn-2 positions (4). The ether bond and the absence of unsaturations in the hydrophilic tail of the phospholipids (and other polar lipids present in archaean membranes).

Abbreviations: BPG, bisphosphatidylglycerol (diphytanylglycerol ether analog); Hbt. salinarum, Halobacterium salinarum; MALDI-TOF/MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; PG, phosphatidylglycerol (diphytanylglycerol ether analog); PGP-Me, phosphatidylglycerophosphate methyl ester (diphytanylglycerol ether analog); PGS, phosphatidylglycerol sulfate (diphytanylglycerol ether analog); PM, phosphatidylglycerolphosphate methyl ester; PPG, phosphatidylglycerol (diphytanylglycerol ether analog); PS, phosphatidylserine (diphytanylglycerol ether analog); S-1Glc, 1-

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This work was supported by Ministero Italiano dell’Università e della Ricerca (Fondi Ateneo), Ministero Italiano della Difesa (Contract No. 1999 and Regione Puglia (Grant code 15, Sens&MicroLab). Manuscript received 2 April 2010 and in revised form 9 June 2010. Published, JLR Papers in Press, June 9, 2010 DOI 10.1194/jlr.D007328

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2818 Journal of Lipid Research Volume 51, 2010
Materials

DNase I was obtained from Sigma Aldrich. 9-Aminoacrididine hemihydrate was purchased from Acros Organics (Morris Plains, NJ). Archaeabacterial lipid standards were isolated and purified from archaeons cultured in our laboratory as described in the following sections. The following archaeabacterial lipids (i.e., diphytanylglycerol ether analogs) were used in the present study: PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerophosphate methyl ester; PGS, phosphatidylglycerosulfate; BPG, bisphatidylglycerol; STGD-1, (3′-sulfogalactobiose)-(6-deoxy-β-D-glucopyranosyl)-2-O-phytanylglycerol; and STGD-1-PA, (3′-sulfogalactobiose)-(6-deoxy-β-D-glucopyranosyl)-2-O-phytanylglycerol-1′-[sn-2,3-di-O-phytanylglycerol], all obtained from Hbt. salinarum. S-DGD-5-PA, (2′-sulfo)mannobiose-2-O-phytanylglycerol, was isolated from a Halorubrum sp. (MdS1 strain). The commercial glycerophospholipids (used as standards)—1,1′,2,2′-tetradecanoyl cardiolipin; 1,1′,2,2′-tetra-(9Z-octadecenoyl) cardiolipin; 1,2-ditetradecanoylsn-glycero-3-phospho; 1,2-ditetradecanoylsn-glycero-3-phospho(1′-rac-glycerol); and 1,2-ditetradecanoylsn-glycero-3-phospho-L-serine; 1,2-di-(9Z-heptadecenoyl)-sn-glycero-3-phosphoethanolamine—were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All organic solvents used in extraction and MS analysis were commercially distilled and of the highest available purity and were purchased from Sigma Aldrich, J. T. Baker, or Carlo Erba. TLC plates (TLC Silica gel 60 A, glass plates), obtained from Merck, were washed twice with chloroform/methanol (1:1, v/v) and activated at 120°C before use.

EXPERIMENTAL PROCEDURES

Isolation and lyophilization of archaeabacterial membranes

Purple membranes were isolated from the high-producing BR strains of Hbt. salinarum as previously described (11) and when indicated were used in lyophilized form. The red membranes of the extremely halophilic microorganism MdS1 were isolated as previously described (13) and lyophilized before lipid analysis.

Lipid extraction of the purple membrane

Total lipids of the PM were extracted using the Bligh and Dyer method (14), as modified for extreme halophiles (15). The extracts were carefully dried under N2 before weighing, and then dissolved in chloroform (10 mg/ml).

Thin-layer chromatography

Total lipid extracts were analyzed by TLC (Merck 10 × 20 cm × 0.25 mm thick layer) with Solvent A (1:1 chloroform/methanol). Lipid detection was carried out by spraying with 5% sulfuric acid in water, followed by charring at 120°C for 45 min.

Isolation and purification of individual lipids from the total extract

The lipidic components of PM were separated by preparative TLC (Merck 20 × 20 cm × 0.5 mm thick layer) in Solvent A. The lipidic components were visualized by staining with iodine vapor and were eluted and recovered from the scraped silica as previously described (15). Isolated and purified phospholipids were dissolved in chloroform at the concentration of 1 mg/ml; glycolipids and cardiolipin analogs in the mass range 1000–2000 amu were resuspended at the final concentration of 2 mg/ml.

Preparation of lipid samples in solution for MALDI-TOF/MS

Samples were prepared as previously described (2). Briefly, the total PM lipid extract (10 mg/ml) and individual lipid components (1–2 mg/ml) were diluted from 20 to 200 µl with isopropanol/acetonitrile (60/40, v/v). Next, 10 µl of diluted sample was mixed with 10 µl of 9-aminoacrididine (10 mg/ml; dissolved in isopropanol/acetonitrile (60/40, v/v)). Then 0.25 µl of the mixture was spotted on the MALDI target (Micro Scout Plate, MSP 96 ground steel target).

Sample preparation of lyophilized archaeabacterial membranes for MALDI-TOF/MS

The procedure for sample preparation is analogous to the preparation of KBr samples for infrared analysis (16, 17). Lyophilized archaeabacterial membranes (PM and red membrane of MdS1) and dry 9-AA matrix were mixed in 2:1 ratio (w/w). Lyophilized membranes were ground with 9-AA in an agate mortar, and the powder mixture was crushed in a mechanical die press to
form a thin pellet disc (less than 1 mm thickness). Small pieces of the disc were then fixed to the MALDI target with double-sided adhesive tape and directly analyzed.

MALDI-TOF/MS

MALDI-TOF mass spectra were acquired on a Bruker Microflex mass spectrometer (Bruker Daltonics, Bremen, Germany). The system utilizes a pulsed nitrogen laser, emitting at 337 nm; the extraction voltage was 20 kV. 600 single laser shots were averaged for each mass spectrum. In the analysis of lipids in solution, the spectrum was obtained by moving the laser within the spot. The laser fluence was kept at about 80% of maximum value to have a good signal-to-noise ratio. At variance in the analysis of lipids in intact membranes, it was not necessary to move the laser during the acquisition of the spectrum, thanks to the thickness of the pellet (see below). It was necessary to keep the laser fluence at 100% for good desorption and ionization of the sample in the pellet; at lower laser intensity, it was not possible to have good signals. All spectra were acquired in negative ion mode using the delayed pulsed extraction. Spectral mass resolutions and signal-to-noise ratios were determined by the software for the instrument, “Flex Analysis 3.0” (Bruker Daltonics). When indicated, spectra were acquired with a Bruker Autoflex mass spectrometer (Bruker Daltonics) in reflector mode; the laser fluence was kept about 10% above threshold to optimize the signal-to-noise ratio.

A mix containing: 1,1',2,2'-tetratetradecanoyl cardiolipin, 1,1',2,2'-tetra-(9z-octadecenoyl) cardiolipin, 1,2-ditetradecanoylsn-glycero-3-phosphate, 1,2-ditetradecanoylsn-glycero-3-phospho-(1'-rac-glycerol), 1,2-ditetradecanoylsn-glycero-3-phospho-L-serine, 1,2-di-(9z-hexadecenoyl)-sn-glycero-3-phosphoethanolamine and the archaebacterial glycolipid (2'-sulfo)Manpα1-2Glc pα1-1-[sn-2,3-di-O-phytanylglycerol] (S-TGD-1-PA) was always spotted next to the sample as external standard. An external calibration was performed before each measurement.

RESULTS

Application of MALDI-TOF/MS to the analysis of archaebacterial lipids in solution

Phospholipids (diphytanylglycerolphosphate ether analogs). The MALDI-TOF mass spectra of some archaebacterial phospholipids present in the membranes of the extremely halophilic archaecal microorganisms are reported in Fig. 1. In particular the following lipids were analyzed: PG, PGP-Me, PGS, and BPG. The chemical structures of analyzed phospholipids are illustrated at the bottom of the Fig. 1. All these archaebacterial phospholipid standards have been isolated and purified from the lipid extract of Hbt. salinarum. PGP-Me is the major phospholipid in all extreme halophiles and extreme haloalkalophiles as well (4). BPG is the archaebacterial glycolipid (2'-sulfo)Manpα1-2Glc pα1-1-[sn-2,3-di-O-phytanylglycerol] (S-TGD-1-PA) that, having two diphytanylglycerol moieties, can be considered a dimeric phospholipid. The structure of S-TGD-1-PA was first determined in our laboratory by combining TLC, NMR, ESI-MS, and MS/MS analysis of the isolated and purified glycolipid and its hydrolysis products (15). It has also been shown that S-TGD-1 is the precursor of S-TGD-1-PA (20).

The lipid extract of the purple membrane of Hbt. salinarum. The lipid extract of the purple membrane was analyzed in parallel by TLC and MALDI-TOF/MS. The names of individual lipid components in the total lipid extract of PM are reported on the side of TLC plate in Fig. 3A, together with the monoisotopic molecular masses obtained by MALDI-TOF/MS analysis. In Fig. 3B, the MALDI-TOF/MS profile of the total lipid extract of purple membrane is reported. The signals in the mass spectrum have been assigned to the various lipids in the extract on the basis of MALDI-TOF/MS analysis of individual archaebacterial lipid standards previously analyzed. Besides the signals of molecular ions of the main lipid species present in the total lipid extracts, signals arising from molecular fragmentation are present. The signal at m/z 1218.1 is attributable to the main glycolipid of purple membrane S-TGD-1, while the peak at m/z 1379.6 corresponds to the minor glycolipid S-TeGD containing four sugar units in the polar head. The glycosylated cardiolipin named S-TGD-1-PA is represented by the signal at 1932.8 ([M+H]⁺). The signal of the phospholipid PGP-Me is at m/z 900.0 ([M-H]⁻), of PGS at m/z 886.0, of PG at m/z 805.9;
Lipid mass spectrometry analysis of intact archaebacterial membranes

MALDI-TOF lipid analysis of equimolar mixtures of archaebacterial phospholipids, including BPG, showed that the BPG signal is always very small compared with those of other phospholipids (data not shown). Lipid analysis of intact lyophilized archaebacterial membranes dry mixed with 9-aminoacridine

Recently MALDI-TOF/MS analysis of insoluble organic compounds of high molecular weight has also been performed by dry mixing the analyte and matrix, and then crushing it in a mechanical die press to form a thin disc or pellet that can be directly scanned by the MALDI laser beam (16, 17). We tested the possibility of applying this approach to the direct lipid analysis of isolated membranes in lyophilized form, thus avoiding the steps of membrane disruption, lipid solubilization, and phase partition.

It has been shown that BPG is only a minor lipid component of PM, likely a contaminant, while S-TGD-1-PA is present in stoichiometric ratio 1:1 with bacteriorhodopsin (9).

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Lipid analysis of intact lyophilized archaebacterial membranes dry mixed with 9-aminoacridine

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It is well known that the purple membrane of Hbt. salinarum has an extraordinary thermal and chemical stability; we have verified that is possible to keep PM in lyophilized form at room temperature for months without changing their lipid composition (unpublished observations).

Lyophilized purple membranes were finely mixed with the powder of 9-AA. The dry mixture was kept under high

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**TABLE 1.** Detected MALDI-TOF/MS ion peaks of archaebacterial lipids of purple membrane

| Lipid Species | Molecular Formula | Theoretical | Experimental |
|---------------|-------------------|-------------|--------------|
| BPG           | C_10H_26O_5P_2    | 1520.3      | 1520.5       |
| PG            | C_6H_12O_5P       | 805.7       | 805.9        |
| PGS           | C_6H_12O_7PS      | 885.6       | 886.0        |
| PGP-Me        | C_6H_12O_7P_2     | 899.7       | 900.0        |
| S-TGD-1       | C_6H_12O_7P_2     | 1217.8      | 1218.1       |
| S-TGD-1-PA    | C_6H_12O_7P_2     | 1992.4      | 1992.8       |

BPG, bisphosphatidylglycerol; GlyC, glycerocardiolipin; PG, phosphatidylglycerol; PGP-Me, phosphatidylglycerophosphate methyl ester; PGS, phosphatidylglycerosulfate; PM, purple membrane; S-TGD-1, (3′-sulfO)Galβ1-6Manβ1-2Glcα1-1-[6-2,3-di-O-phytanylglycerol]; S-TGD-1-PA, (3′-sulfO)Galβ1-6Manβ1-2Glcα1-1-[6-2,3-di-O-phytanylglycerol]-6-[phospho-α,β-phospho-2,3-di-O-phytanylglycerol].

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**Fig. 1.** MALDI-TOF MS analysis of phospholipids standards (diphytanylglycerolphosphate ether analogs) isolated and purified from extremely halophilic archaebacterial microorganisms. Shown are the peaks of the molecular ions [M-H]: BPG at m/z 1520.5 (A); PGP-Me at m/z 900.0 (B); PGS at m/z 886.0 (C); and PG at m/z 805.9 (D). In addition to the peaks of the molecular ions, some fragments that were produced during the desorption/ionization process are shown: the fragment at m/z 731.7 corresponding to PA is present in all spectra, while the fragment at m/z 805.9 in the PGS spectrum (C) corresponds to PG. Under the spectra, chemical structures of the archaebacterial phospholipids are reported.

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Finally, the small peak at m/z 731.7 corresponds to PA, a lipid fragment. At variance from ESI-MS lipid profile, in MALDI-TOF mass spectrum the peak of BPG can be barely detected by enlarging the y axis, although it gives a good signal when analyzed individually (see mass spectrum in Fig. 1).

It has been shown that BPG is only a minor lipid component of PM, likely a contaminant, while S-TGD-1-PA is present in stoichiometric ratio 1:1 with bacteriorhodopsin (9).
membranes in lyophilized form was also verified. Fig. 6 illustrates the MALDI-TOF/MS analysis of lyophilized red membranes isolated from the strain MdS1, another extremely halophilic microorganism of the Archaea world. MdS1 red membranes are highly enriched in BPG (12, 13).

The lyophilized MdS1 red membranes and dry 9-AA were mixed in 2:1 ratio, obtaining a thin red pellet. A fragment of the pellet was used for MALDI-TOF/MS analysis. The resulting MALDI-TOF/MS lipid profile was perfectly superimposable onto the lipid profile obtained by previous TLC and ESI-MS analysis (12, 13). Fig. 6A reports the TLC lipid profile of red membranes and lipid assignments based on data in previous literature and mass spectrometric analysis. Experimental lipid masses obtained by MALDI-TOF/MS analysis correspond well to calculated lipid masses and to those previously obtained by ESI-MS analysis (12, 13). It can be seen that in the MALDI spectra of membrane/matrix pellets most of archaeabacterial lipids are represented by their sodium adducts. The excellent quality of the MALDI-TOF/MS spectrum of lyophilized MdS1 membranes in Fig. 6 is likely due to the optimal homogeneity of the red membranes/9-AA pellet.

A representative MALDI-TOF mass spectrum obtained by analyzing the PM/9-AA pellet is reported in Fig. 5. The MALDI-TOF/MS lipid profile obtained in these experimental conditions corresponds quite well to the one reported in Fig. 3 that was obtained by analyzing the PM lipid extract. The glycosylated cardiolipin S-TGD-1-PA is represented by the signal at m/z 1932.8, corresponding to the fragment [M-HSO₃]⁻ produced during the desorption/ionization process. Under the spectra, the previously determined (7, 15) chemical structures of the archaeabacterial glycolipids are shown.

Fig. 2. MALDI-TOF/MS analysis of glycolipids standards (diphytanylglycerol ether analogs) isolated and purified from extremely halophilic archaeal microorganisms. The peaks of the molecular ions [M-H]⁻ of S-TGD-1 (B) and S-TGD-1-PA (A) are at m/z 1218.1 and m/z 1932.8, respectively. Present in the spectrum of S-TGD-1-PA (A) is a peak at m/z 1853.0, corresponding to the fragment [M-HSO₃]⁻ produced during the desorption/ionization process. Under the spectra, the previously determined (7, 15) chemical structures of the archaebacterial glycolipids are shown.
Lipid mass spectrometry analysis of intact archaebacterial membranes

Density to the halophile membranes. No nitrogenous base-containing phospholipids, such as phosphatidylserine or phosphatidylethanolamine, are present in extreme halophiles. This may be characteristic of the halophilic Archaea, in contrast to the methanogenic and thermophilic Archaea. Information on lipid biosynthesis in Archaea is available thanks to labeling studies with whole cells \((5, 22)\).

In a previous study, we analyzed the lipids of the purple membrane by combining TLC and NMR analysis. The It is noteworthy that the BPG signal at \(m/z\ 1542.9\) is comparable to that of other membrane lipid components; therefore, the novel method of lipid analysis offers the advantage of overcoming the problem of difficult ionization of BPG when it is mixed with other phospholipids in solution.

**DISCUSSION**

Recent applications of MALDI-TOF/MS to the analysis of cellular lipids have shown the potential of a technique long considered of practical utility only for protein analysis \((2, 21)\).

It has been shown that MALDI mass spectrometry allows the analysis of glycerolipids, glycerophospholipids, triglycerides, cholesterol, and cholesterol derivatives, while reports describing the application of MALDI to the analysis of archaebacterial lipids are not yet available.

In this study, we illustrate for the first time the application of MALDI-TOF/MS to the analysis of archaebacterial lipids in solution, and we show that it is possible to directly analyze lipids of membranes isolated from archaean microorganisms without prior extraction/separation steps.

We have analyzed archaean membrane lipid standards and the total lipid extract of the archaean membranes isolated from halophilic microorganisms, obtaining results comparable with those obtained by ESI-MS analysis.

Most of the phospholipids and glycolipids of extreme halophilic archaeanos are anionic, so that their negatively charged groups would impart a high negative charge density to the halophile membranes. No nitrogenous base-containing phospholipids, such as phosphatidylserine or phosphatidylethanolamine, are present in extreme halophiles. This may be characteristic of the halophilic Archaea, in contrast to the methanogenic and thermophilic Archaea. Information on lipid biosynthesis in Archaea is available thanks to labeling studies with whole cells \((5, 22)\).

In a previous study, we analyzed the lipids of the purple membrane by combining TLC and NMR analysis.

**Fig. 3.** A: TLC lipid profile of the total lipid extract of purple membranes of *Halobacterium salinarum*. Lipid abbreviations and molecular ion masses are reported. Lipid assignments are based on previous studies \((15, 20)\). B: MALDI-TOF mass spectrum of the total lipid extract of purple membranes of *Hbt. salinarum* acquired in the negative ion mode using 9-AA as the matrix.

**Fig. 4.** Photograph of fragments of PM/9-AA pellet attached to the MALDI-TOF target. Dimensions of the target are approximately \(53 \times 41\) mm. MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; PM, purple membranes; 9-AA, 9-aminoacridine.
results obtained allowed the estimation of stoichiometric ratio of individual lipid component and the sole protein of the purple membrane bacteriorhodopsin (9).

In this study, we considered the purple membrane as a model membrane to investigate the potential of MALDI-TOF/MS in the analysis of lipids of archaeal microorganisms. Results indicate that 9-AA in solution is a suitable matrix for the desorption/ionization of archaeal phospholipids and glycolipids. The method, originally developed by Sun G. et al. (2), allows good ionization and easy detection of the dimeric archaeal phospholipids or cardiolipins. The sensitivity of the method is particularly high for the archaeal sulfated glycolipids, which in analogy with sulfatides of eukaryotic cells (23), exhibit an extremely high tendency to ionize in the presence of 9-AA.

The MALDI-TOF/MS profile of the lipid extract of the purple membranes contains the same main signals present in the ESI mass spectrum (15). All the lipids in the extract can be easily revealed by this technique of analysis, with the exception of the minor PM lipid component BPG, whose signal is very low.

Interestingly we also show that it is possible to perform a direct lipid analysis of the intact lyophilized archaeabacte-
rrial membranes by MALDI-TOF/MS dry mixed with the matrix. We recommend care in the preparation of the membrane/matrix pellets, because the incomplete homogeneity of the samples might cause some inaccuracy in the measurements.

The study of lipid composition of cell membranes normally requires two separate steps: 1) extraction of lipids by harsh organic solvents and 2) combined chromatographic and mass spectrometry analysis. The novel approach to analyze lipids of intact membranes is particularly useful if doubts about the presence of artifacts introduced by the lipid extraction procedure are raised (e.g., highly polar lipids as sulfated polyglycolipids may preferentially partition into the methanol-water phase and therefore be absent in the chloroform phase). In a previous study of $^{31}$P NMR analysis of solubilized purple membrane, the phosphorus signal of the glycosylated cardiolipin S-TGD-I-PA could not be detected, raising doubts about the presence of the archaebacterial cardiolipin in the PM (24). In the present study, our data confirm that archaebacterial cardiolipins are true components of the membrane matrix of the purple membrane.

The direct lipid analysis of lyophilized membranes appears to be of general applicability for archaebacterial lipids, whose structures are chemically stable even in extreme environmental conditions. During the dehydration process, the polar heads of phospholipids lose the associated water, whereas sodium ions likely remain bound to membrane. This might explain why most of highly polar lipids of archaebacterial membranes appear as sodium adducts in the MALDI spectra of lyophilized membranes.

The method of MALDI-TOF/MS lipid analysis of lyophilized membranes is particularly suited to the analysis of the complex archaebacterial phospholipid BPG, allowing an easier detection of it. The sodium adduct of BPG represents one of the main signals in the MALDI-TOF/MS lipid profile in Fig. 6, although it appears difficult to obtain the ionization of BPG in solution in the presence of other phospholipids and glycolipids.

The novel method we describe might be of general applicability. It offers the advantage of quickly gaining information about membrane lipid components without disrupting oraltering the membrane matrix. Experiments are in progress to evaluate the potential of this novel method in the study of lipid composition of bacterial and eukaryotic membranes.14

The authors thank Prof. Francesco Naso for the use of MALDI-TOF/MS instrument Bruker Microflex in the Chemistry Department of the University of Bari, Prof. Loreto Gesualdo for the use of MALDI-TOF/MS instrument Bruker Autoflex in the Proteomic Facility of the University of Foggia, and Dr. Patrizia Lopalco for preparing the archaebacterial lipid standards and membranes.

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