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Fast atom bombardment mass spectrometry as a rapid means of screening mixtures of ether-linked polar lipids from extremely halophilic archaebacteria for the presence of novel chemical structures

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ABSTRACT

Fast atom bombardment mass spectrometry was used to analyse intact polar ether lipids present at microgram levels in crude lipid mixtures extracted from *Halobacterium halobium*, *Natronococcus occultus* and *Halobacterium marismortui*. Negative-ion spectra showed the intact deprotonated lipid molecules and in some instances their sodium salts. The simplicity of the mass spectra permits the rapid screening of polar lipid mixtures for the presence of novel lipids. Additional structural information of ions with selected masses was obtained after collisionally induced decomposition.

INTRODUCTION

Microorganism which grow in extreme environments often possess unique biological molecules which enable them to survive under conditions which would be lethal to other life forms [1]. Once identified and studied, the unique chemical properties of these molecules can be used in many specific biotechnologies. The core of the archaebacterial polar lipids consists of a glycerol backbone with a polar head group linked via the phosphodiester bond to C-3. In contrast to all other biota, the isoprenoidyl side-chains, usually phytanyl chains, are ether-linked to the C-1 and C-2 of the glycerol backbone [2,3].

We describe here how fast atom bombardment mass spectrometry (FAB-MS) can be used to screen rapidly extracts of extremely halophilic archaebacterial cells containing mixtures of ether-linked polar lipids for the presence of novel structures [4,5].

EXPERIMENTAL

Archaebacterial cells were harvested from batch liquid monocultures by centrifugation. Lipids were extracted with dichloromethane–methanol–water (1:2:0.9, v/v) and were separated into three polarity groups by successively eluting

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the lipids from a silica gel column with dichloromethane, acetone and methanol (Fig. 1). Samples of the acetone and methanol eluates were spotted on silica gel thin-layer chromatographic (TLC) plates (Kieselgel 60) and developed in chloroform–methanol–acetic acid–water (85:22.5:10:4, v/v). Regions on the TLC plates containing lipids were rendered visible with UV light and selective staining with rhodamine for all lipids, and acidic molybdate solution for phospholipids and α-naphthol for reducing sugars.

Negative-ion FAB mass spectra were obtained on a Kratos MS 50 RF TC high-field magnet instrument equipped with a Kratos FAB source, using an 8-keV xenon beam (source pressure 10^{-3} Pa). The sample was dissolved in a small amount of methanol and 3-nitrobenzyl alcohol (3-NBA), placed on the steel tip of a sample probe and inserted into the ion source. The spectrum was recorded at an accelerating potential of 8 kV with a magnet scan rate of 30 s/decade at a resolution of 2500. Tandem MS (MS–MS) was performed on a Finnigan MAT HSQ 30 mass spectrometer. Selected ions were collided (45 V) with argon (0.13 Pa) confined in a collision quadrupole and daughter ions were separated by a quadrupole analyser.

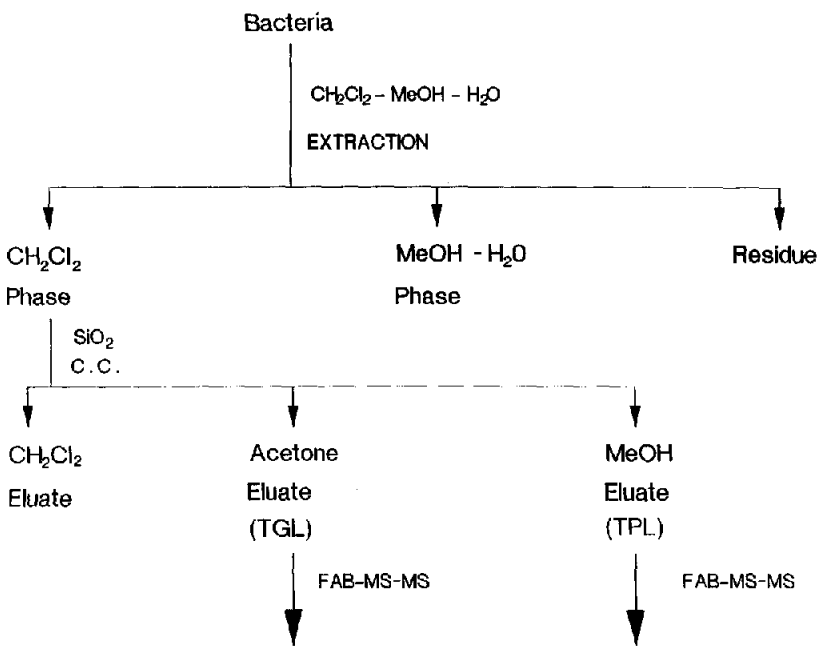

Fig. 1. Analytical scheme. MeOH = Methanol.

RESULTS AND DISCUSSION

TLC of the lipids in the acetone eluate showed that the major lipids stained negative for phosphate and positive for reducing sugars. This eluate is referred to as the total glycolipid (TGL) eluate. Similarly, TLC of the methanol eluate showed that the major lipid components contained phosphate but no reducing sugars. This eluate is referred to as the total phospholipid (TPL) eluate.

Positive- and negative-ion FAB MS and MS–MS were applied to lipid mixtures in both the acetone (TGL) and methanol (TPL) eluates from the silica

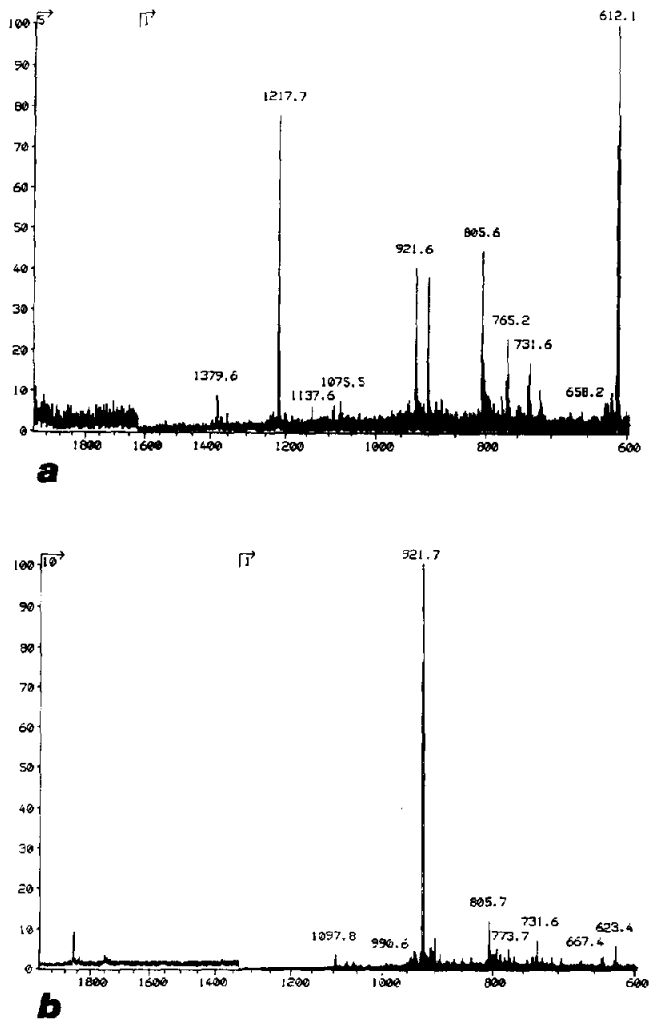

Fig. 2. Negative-ion FAB mass spectra of (a) glycolipids and (b) phospholipids from *Halobacterium halobium*.

column. These eluates contained high concentrations of alkali metal ions because the bacteria require a saturated salt solution for growth and the extraction and purification procedures failed to remove them. The negative-ion FAB mass spectra of the lipids in both fractions were simpler than the corresponding positive-ion mass spectra. In the positive-ion mode each lipid molecule usually produced an ion derived from the protonated molecule and the molecule cationized by Na^+. Additionally, each ionizable group (*i.e.*, phosphate and sulphate) could be cationized by either H^+ or Na^+. Using 3-NBA as the matrix, the negative-ion mass spectra show abundances of ions corresponding to the deprotonated lipid molecules and their deprotonated salts between about 50% (TPL) and 1–5% (TGL) of most abundant matrix ion at *m/z* 153.

The most significant ion apart from the matrix ion at *m/z* 612.2 in the mass spectrum of the TGL fraction of *Halobacterium halobium* (Fig. 2a) is the ion at *m/z* 1217.7. This mass corresponds to the deprotonated sulphated triglycosylated analogue of 2,3-di-O-phytanyl-*sn*-glycerol (S-TGD_{dpe}). The signal of low abundance at *m/z* 1379.6 indicates the presence of a glycolipid containing four sugar entities. The ions at *m/z* 921.6, 899.6, 805.6 and 731.6 result from small amounts of phosphorus-containing lipids, which were probably eluted from the silica column with acetone.

The mass spectrum of the TPL fraction of *Halobacterium halobium* (Fig. 2b) contains a base peak at *m/z* 921.6 which corresponds to the monosodium salt of the diphytanyl ether analogue of the methylated phosphatidylglycerol phosphate (PGP_{dpe}-OCH_3). Ions at *m/z* 805.6 and 731.6 correspond to the deprotonated molecule of diphytanyl ether analogues of phosphatidylglycerol (PG_{dpe}) and of phosphatidic acid (PA_{dpe}), respectively.

The tandem mass spectrum of the ion at *m/z* 1217.7 from the acetone eluate

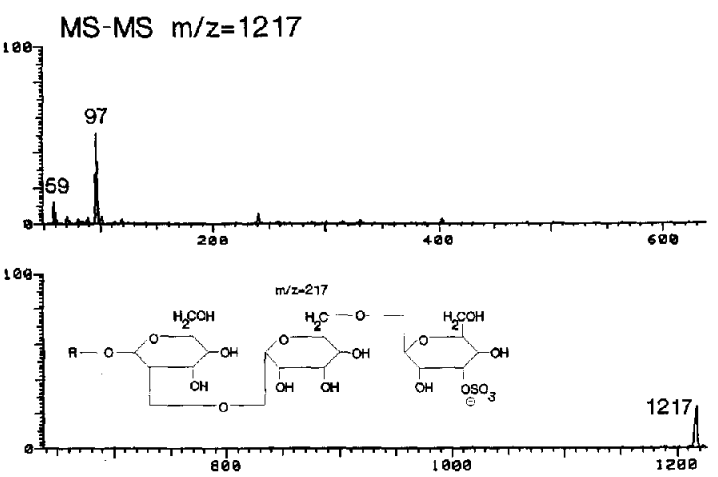

Fig. 3. Negative-ion FAB tandem mass spectrum of *m/z* 1217 of glycolipids from *Halobacterium halobium*.

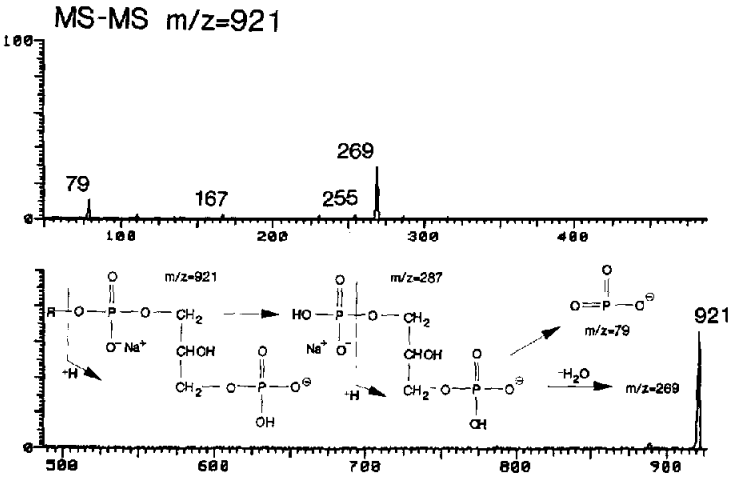

Fig. 4. Negative-ion FAB tandem mass spectrum of *m/z* 921 of phospholipids from *Halobacterium halobium*.

(Fig. 3) shows only one fragment at *m/z* 97, confirming the presence of a sulphate group.

In the MS–MS analysis of the ion at *m/z* 921.7 (Fig. 4) in the methanol eluate two structurally significant ions occur. Cleavage of the ether bond between the glycerol backbone and the phosphate group of the deprotonated monosodium salt results in a negatively charged fragment derived from the polar head containing the monosodium salt (the non-abundant ion at *m/z* 287), which lost water and produced the ion at *m/z* 269. The ion at *m/z* 79 corresponds to the $[PO_3]^-$ group.

Investigation of the TGL fraction of the haloalkaliphilic archaebacterium *Natronococcus occultus* (Fig. 5a) with negative-ion FAB using 3-NBA as the matrix did not show ions from glycolipids. TLC confirmed the absence of glycolipids in this bacterium.

Negative-ion mass spectra of the TPL fraction (Fig. 5b) exhibit two groups of ions differing in mass by 70 Da. This result is due to two phospholopids with different combinations of isoprenoidyl side-chains, a 2,3-di-O-phytanyl-*sn*-glycerol derivative and a 2-O-sesterterpanyl-3-O-phytanyl-*sn*-glycerol derivative. Ions formed from the diphytanyl ether are explained above. Ions at *m/z* 875.6 and 991.6 are explained by the sesterpanyl–phytanyl diether analogues of phosphatidylglycerol (PG_{spe}) and phosphatidylglycerol phosphate (PGP_{spe}-OCH_3).

The mass spectrum of the TGL fraction of polar lipids from *Halobacterium marismortui* (Fig. 6a) show ions attributable to molecular masses only with loss or attachment of an SO_3 group. The origin of the intense ion at *m/z* 867.4 is not apparent. The ions at *m/z* 805.6 and 732.4 again result from small amounts of phosphorus-containing lipids eluted from the silica column with acetone.

The negative-ion mass spectrum derived from the TPL fraction (Fig. 6b) is

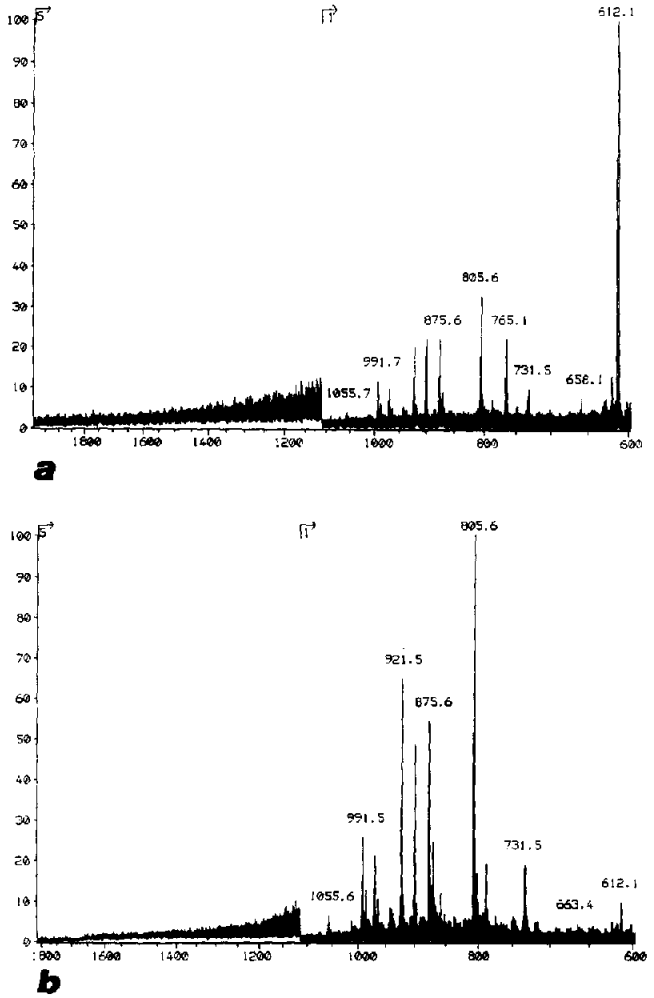

Fig. 5. Negative-ion FAB mass spectra of (a) glycolipids and (b) phospholipids from *Natronococcus occultus*.

similar to that obtained from *Halobacterium halobium*. However, the series of ions at m/z 805.6, 803.5, 801.5, 799.5, 797.5, 795.5 and 793.5 suggest the presence of analogues of PG_{dpc} with unsaturated phytanyl chains (see also Fig. 7).

FAB-MS provided a facile means of detecting the presence of novel polar lipids in crude lipid mixtures. Polar lipids could be tentatively identified on the basis of their molecular weights and their ability to form salts with sodium. This procedure could be further expedited by using a high-performance liquid chromatographic system linked to the mass spectrometer via a continuous-flow FAB interface. However the problem of different molecules with the same mass may be

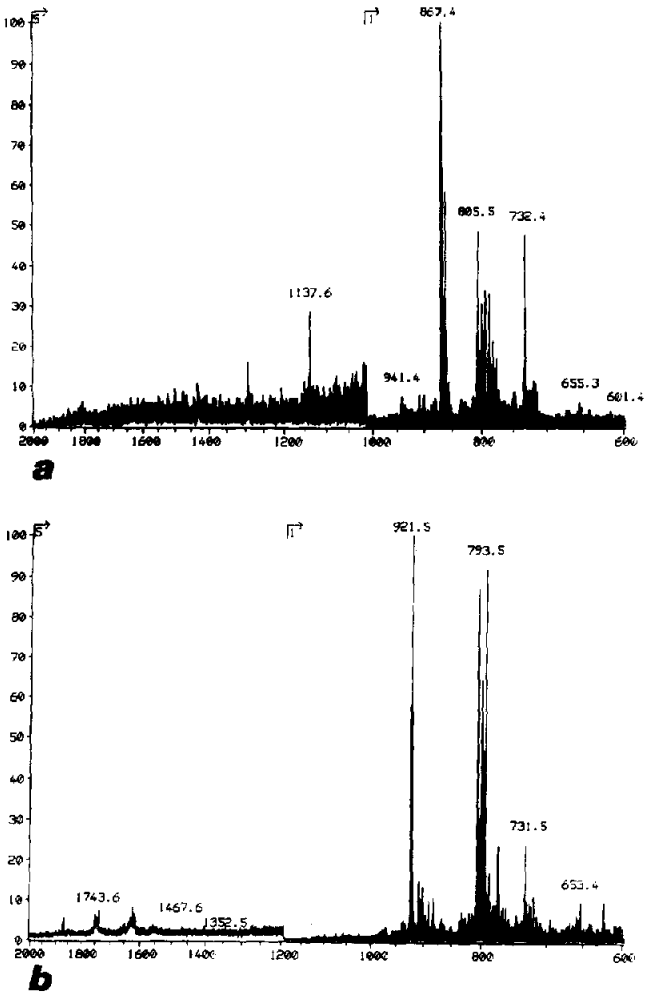

Fig. 6. Negative-ion FAB mass spectra of (a) glycolipids and (b) phospholipids form *Halobacterium marismortui.*

more difficult than first assumed, particularly in the area of natural product analysis. In this study, the inability to resolve phosphate and sulphate esters solely on the basis of mass became apparent. The problem was also encountered in the structural analysis of the saccharide chains of the glycoplipids. However, FAB-MS–MS can provide additional information for determining the structures of microbial polar lipids.

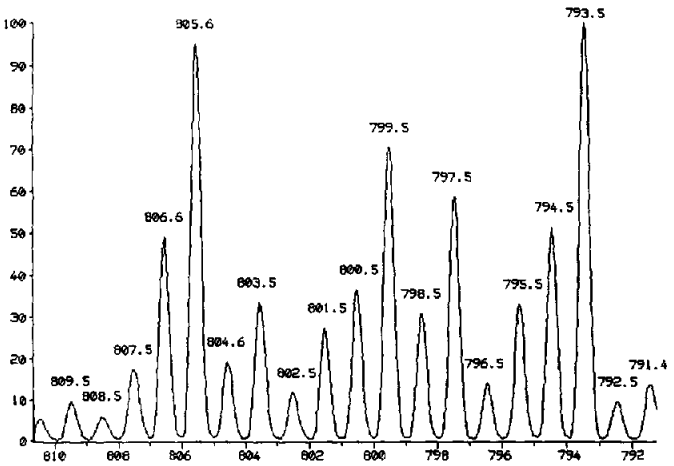

Fig. 7. Mass region *m/z* 790–810 of negative-ion FAB mass spectra of phospholipids from *Halobacterium marismortui*.

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