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# MASS SPECTRAL ANALYSIS OF COMPLEX LIPIDS DESORBED DIRECTLY FROM LYOPHILIZED MEMBRANES AND CELLS*

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Three desorption ionization techniques -- laser desorption, plasma desorption and fast atom bombardment mass spectrometry -- have been applied to lyophilized cells, membranes, lysed cells and various extracts. It has been shown that intact polar lipids are selectively desorbed from biological membranes by these methods and that their mass spectra provide "fingerprints" which reflect the unique biochemical composition of each class of cell or membrane. 

Having noted the preference of desorption methods for preformed organic ions and zwitterions and polar molecules (1), we have considered the possibility that complex polar lipids could be selectively desorbed directly from bacterial and mammalian cells and membranes and analyzed by mass spectrometry, without prior extraction, purification and derivatization. Such analyses might then be used, for example, for chemotaxonomic classification of bacteria, or to distinguish lipids and biochemical processes in the outer layer of the myelin membrane from those of inner layers.

Attempts to classify bacteria by mass spectrometric analysis of intact cells have been reported using pyrolysis mass spectrometry (2,3), and rely on

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pattern recognition, or statistical techniques, rather than identification of specific complex molecules. On the other hand, despite the fact that the relative proportions of lipids are strongly effected by variations in growth media, age, pH, growth temperature and aeration, specific lipid content has been shown to correlate well with taxonomic grouping (4,5). Thus, it was felt that the presence of molecular ions in the mass spectrum which correspond to specific lipids would provide different information than a distribution of pyrolytic products, and that selectivity might be obtained which could reflect the chemical nature of the compounds desorbed, the biological matrix in which they are imbedded, and the specific desorption method used. For the last reason we employed several ionization techniques in our initial attempts to examine the possibilities for selective desorption of intact lipids.

## EXPERIMENTAL

Three desorption ionization methods were used. Positive and negative ion plasma desorption mass spectra (PDMS) were obtained on a BIO-ION Nordic (Uppsala) BIN-10K mass spectrometer with a 10 μCi Cf-252 source. Positive ion laser desorption mass spectra (LDMS) were obtained using a CVC Products (Rochester, NY) model 2000 time of flight mass spectrometer and a Tachisto (Needham, MA) 215G carbon dioxide laser (6,7). Positive and negative ion fast atom bombardment (FAB) mass spectra were obtained on a Kratos (Ramsey, NJ) MS-50 double-focussing mass spectrometer.

Myelin was prepared and purified from young adult rat brain according to the procedure of Norton (8).

A genetically disabled E. coli strain, L-600, was grown in a complex LB broth for 12 hours at 37°C with agitation. The culture broth was then centrifuged and the supernatant removed. The cells were washed 3 times with distilled water, lyophilized and stored at -80°C. Samples of lysed cells were prepared by treating lyophilized cells with 1 ml of MeOH:$CHCl_3$ (2:1) for 30 minutes. The solvent was blown off with $N_2$, and distilled water was added to make a known concentration of 10-20 μg cells per μl $H_2O$.

Lyophilized cells and membranes and lysed cells were added in water suspensions directly to the probe tip (for LDMS and FAB) or to the aluminum sample foil (in PDMS). Samples for LDMS were doped with potassium chloride, while those for FAB were mixed on the probe tip with a drop of thioglycerol (positive spectra) or diethanolamine (negative spectra).

## RESULTS AND DISCUSSION

Myelin is a multilayered membrane which forms a sheath around the axons of nerve cells. It is particularly rich in cerebrosides and sulfatides, lipids not found in other parts of the body in more than trace quantities. Figure 1, shows the laser desorption mass spectrum of lyophilized rat brain myelin, and peaks which are characteristic of molecular ions $(M+K)^+$ of

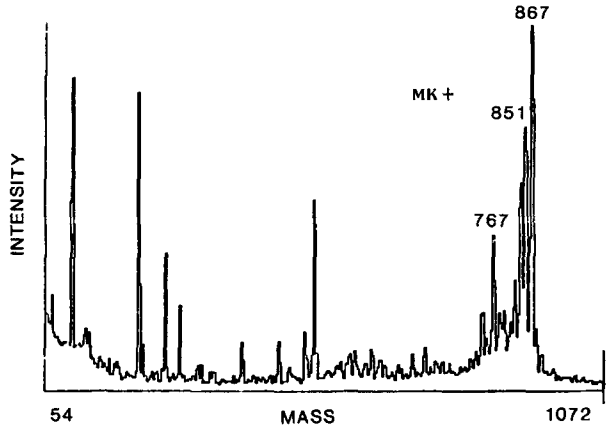


FIGURE 1. Laser desorption mass spectrum of lyophilized rat brain myelin.

cerebrosides. Sulfatides, on the other hand, were easily observed in the negative ion PDMS spectra (Figure 2.) as $(M-H)^-$ ions. Interestingly enough, cerebroside ions were not observed in positive ion FAB or PDMS spectra. Rather, ions characteristic of phosphatidylcholine (pc) dominated these spectra.

Polar lipids could also be desorbed directly from lyophilized bacterial cells. The positive ion FAB mass spectrum of E. coli in Figure 3. shows ions ($MH^+$ at 705 and 733 amu; $MNa^+$ at 727 and 755 amu) which correspond to intact phosphatidylethanolamines (PE) containing 16:0 and 18:0 fatty acids. Fragment ions of 524 and 552 amu are formed by loss of the phosphoethanolamine group. PE are the dominant phospholipid in E. coli and

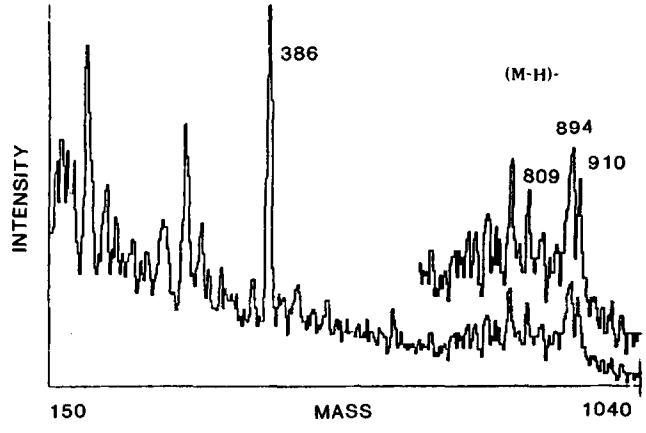


FIGURE 2. Negative ion plasma desorption mass spectrum of lyophilized rat brain myelin.

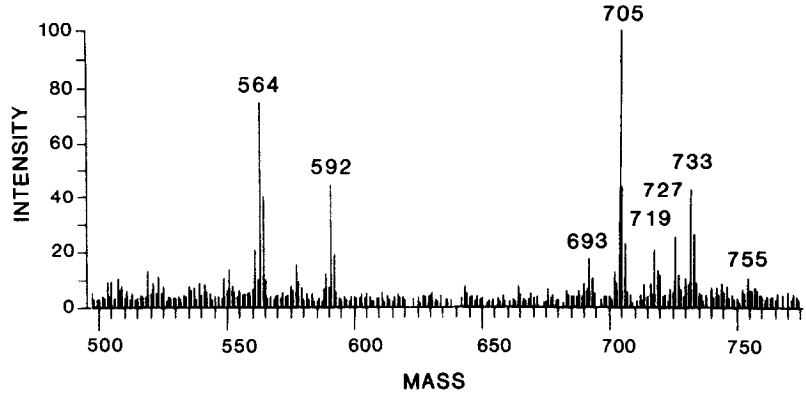


FIGURE 3. Fast atom bombardment mass spectrum of lyophilized E. coli cells.

are observed as well by LDMS, but as $MK^+$ ions. The detection limit for LDMS was 2 μg of cells, of which about 5% is PE, based on dry weight (9). Ions corresponding to PE were not observed from lyophilized E. coli cells, however, using PDMS, suggesting that the high flux beams employed in FAB and LD serve as well to disrupt the cells. Consequently, solvent-lysed cells were also examined. In Figure 4, the laser desorption mass spectrum of solvent-lysed E. coli show the characteristic ($MK^+$) molecular ions at 743 and 771 amu, corresponding to PE.

In general, disruption of the cells significantly increases the intensity of phopholipid signals. Either lysing the cells with solvent or agitating them with glass beads prior to mass spectral analysis increased the PE signal intensity three-to five-fold in the FAB mass spectrum. In the PDMS

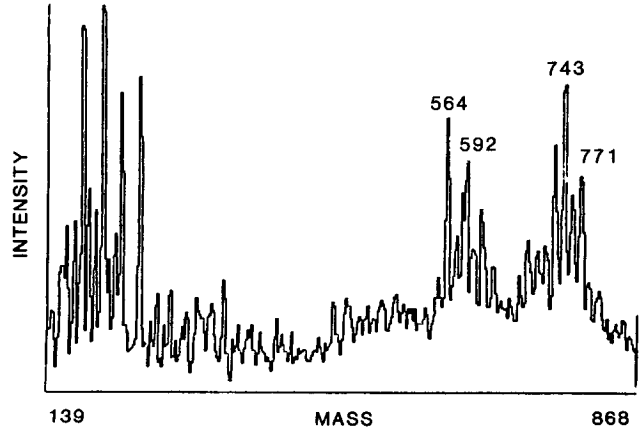


FIGURE 4. Laser desorption mass spectrum of solvent-lysed E. coli cells.

analysis, lysing was necessary for observation of phospolipid ions, while preparation of a $CHCl_3$/MeOH lipid extract improved the quality of the spectra even further. Interestingly, the PE signal intensity from a total lipid extract, about 9% of the cell's dry weight (9), was only about 10% greater than the PE signal from lysed whole cells in the FAB case, indicating significant selectivity in the desorption of phospholipids over other cell components.

Preliminary analyses of solvent-lysed Bacillus subtilis revealed ions corresponding to phosphatidylglycerol, diglycerides and diglucosyldiglycerides in the FAB and LDMS spectra.

We note that other investigators have reported desorption mass spectra obtained directly from complex biological matrices. Cooks and coworkers (10) have reported detection of quaternary ammonium alkaloids in the analysis of lyophilized mushrooms by secondary ion mass spectrometry (SIMS) and direct chemical ionization (DCI). Ross, et.al. (11) have reported negative ion FAB mass spectra of intact algae cells, which contained peaks corresponding to the carboxylate anions of individual fatty acids. Our present method provides information not only on fatty acid distribution, but also on intact complex lipids, such as glycosphingolipids (cerebrosides and sulfatides) and phospholipids (PE, PC, etc.). The selective desorption of preformed ions from complex matrices demonstrated in these papers is here extended to families of complex lipids, some of which are ionic. These offer a unique window on membrane biochemistry and have high potential as biomarkers for chemotaxonomy.

## CONCLUSIONS

We have demonstrated that a variety of complex polar lipids can be selectively desorbed from intact mammalian and bacterial membranes and cells, and which reflect and distinguish the biochemistries of these systems. We note that different desorption techniques select for different biomarkers in some cases. We are now attempting to understand the mechanisms of such selectivity, the possibilities for characterizing and distinguishing

bacterial and mammalian cells, and the possibilities for studying disease and diet induced biochemical changes in mammalian cells.

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