Structural characterization of neutral and acidic glycolipids by TLC/VC-MALDI-FTICR MS and MS/MS

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Novel Aspect

The structures of lipids from human milk desorbed directly from TLC plates were analyzed by SORI-CAD and IRMPD-FTICR MS.

Introduction

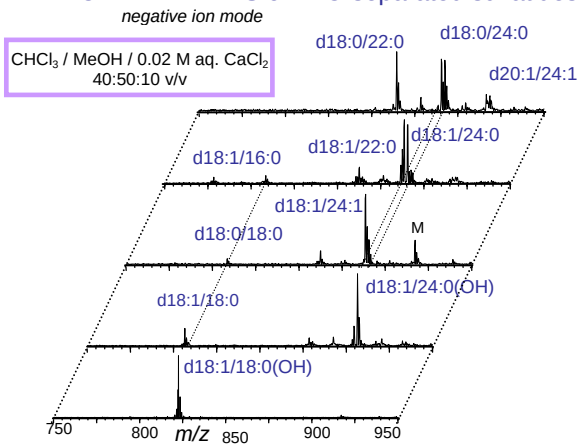
Glycosphingolipids (GSLs) and phospholipids (PLs) are components of cell membranes that regulate their biophysical properties and participate in diverse biological processes. Sulfatides from human milk, but not those from bovine brain, are active against HIV infection of human macrophages and lymphocytes. The biological roles depend upon the specific oligosaccharide and the ceramide moieties. PLs have polar headgroups of ethanolamine, choline, serine, inositol, and phosphatidylglycerol. The acyl groups may differ in their degree of saturation/hydroxylation and in their fatty acyl chain length. Here, we use vibrationally cooled MALDI-FTICR MS for the detection of TLC-separated species followed by their fragmentation by SORI-CAD and IRMPD to determine structural details.

Methods

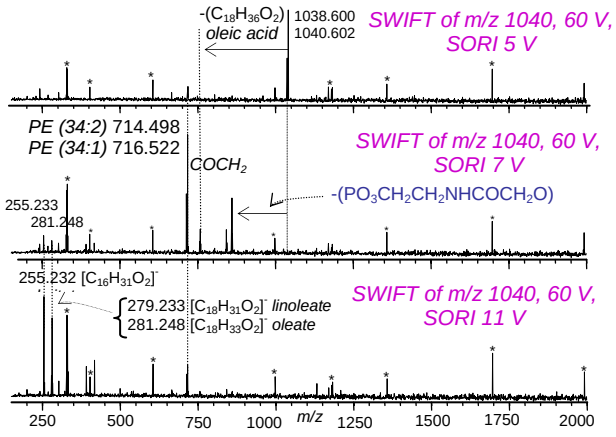
Commercial lipid standards or lipids extracted from pooled human milk were separated by TLC using chloroform/methanol/acetic acid/water (65:25:3:1 v/v); the plate was then sprayed with the saturated matrix solution, affixed to the target, and scanned by VC-MALDI-FTICR MS. Fragmentation was performed by SORI-CAD and IRMPD techniques. We have previously described ganglioside separations and instrumental parameters for VC MALDI-FTICR MS, from standard targets [1] and from TLC plates [2]. In this study, the lipids were MALDI-desorbed directly from TLC plate surfaces with ~0.5 -mm sampling steps and thermalized by the cooling gas. The peaks of interest were isolated by SWIFT, if necessary, and fragmented by SORI-CAD and IRMPD. The results were compared to glycolipid fragmentation on nanospray ESI-FTICR MS.

Preliminary results

VC-MALDI-FTMS of TLC-separated sulfatides



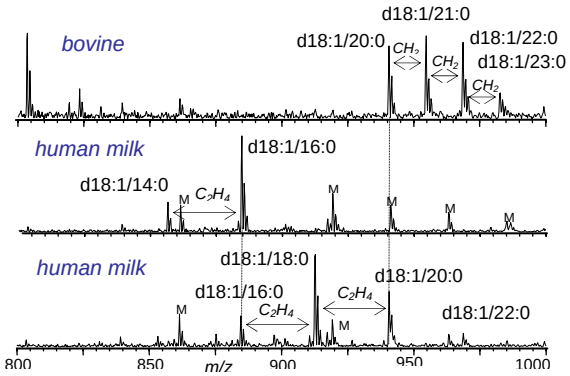
VC-MALDI-FTMS of TLC-separated phospholipids



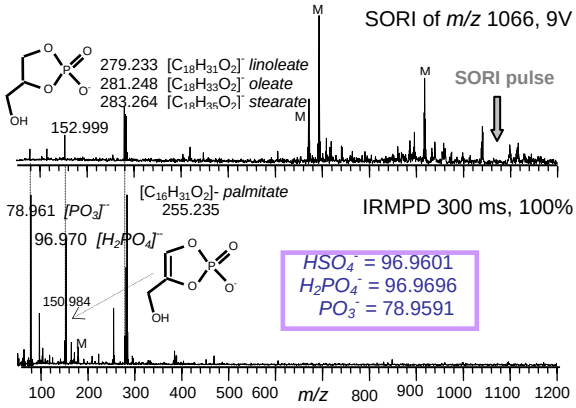
Numerous neutral and acidic GSL and PL homologs were found following each scanning step. Short IRMPD pulses defined the glycan moiety and higher energy irradiation established the ceramide structures. Application of SORI-CAD and IRMPD to the sulfatides resulted in high-abundance peaks characteristic of HSO_4^- and hexose sulfate. Fatty acyl chain lengths varied (C16 - C24); some were hydroxylated. Due to the collisional cooling, the intact sulfatides could be detected in both +/- ion modes. Evidence for the presence of a phospholipid was inclusion of *m/z* 78.959 $(PO_3)^-$, *m/z* 96.970 $(H_2PO_4)^-$, other characteristic products at *m/z* 152.999 and *m/z* 150.984 (IRMPD only), following decomposition of the $[M-H]^-$ and $[M-15]^-$ ions. Compared to the SORI-CAD spectra, the IRMPD spectra are easier to interpret; they demonstrate a higher fragmentation efficiency, and are free from "blind spots".

Four major species desorbed from a single TLC spot produced (+) mode spectra showing sequential elimination of two oleoyl groups; (-) mode MS obtained from these spots showed evidence for loss of C16:0, C18:0, C18:1 and C18:2 acyl chains. Analysis of spots with lower R_f values by SORI-CAD indicated the presence of a glycerophospholipid with palmitoyl and oleoyl (34:1) or palmitoyl and linoleoyl (34:2) substituents, and spots at high R_f had linoleoyl and stearoyl (36:2) and linoleoyl and oleoyl (36:3) substituents. Other unusual phosphatidyl derivatives were present; ongoing experiments should define their structures and sources.

VC-MALDI-FTMS of TLC-separated lactoceramides



SORI-CAD *vs* IRMPD of the TLC-separated phospholipid fraction



Acknowledgements

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References

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