

Exploring the lipidome of infected *Emiliana huxleyi*

The use of mass spectrometry for the structural elucidation of membrane lipids implicated in viral invasion (Chemical fortifications or a chink in the armor of *Emiliana huxleyi*?).

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Introduction

Using *mass spectrometry* to examine the total lipid extract of Ehux374 *before, during and after infection* by coccolithovirus EhV86 we have been able to identify several new, unreported glycosphingolipids (GSLs) and a glycerolipid that *appear or change in composition during infection*, as compared to resistant strain Ehux373. Check out companion poster by Jamey Fulton for discussion of the significance of these lipids.

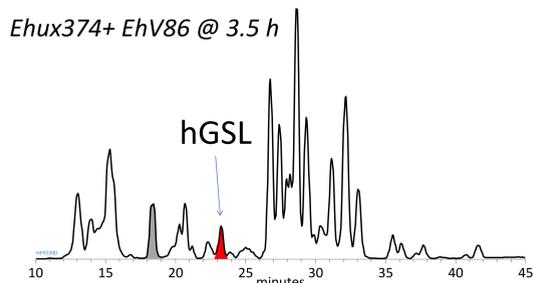
Methodology

Ehux374 & 373 cultures were infected with EhV86 and sampled at regular intervals. Total lipid extracts were analyzed by *normal phase-HPLC-electrospray/ion trap-MS*. Lipids of interest were isolated using *prep-scale HPLC*. Long-chain bases (LCBs) were prepared by alkaline hydrolysis and

TMS derivatives of vGSLs were prepared by derivatization with BSTFA with 1% TMCS. Accurate mass data was acquired for molecular ions and product ions in both positive and negative ion mode using *Fourier transform – ion cyclotron resonance MS (FT-MS)*. Where possible, standards were used for comparison and confirmation of structures.

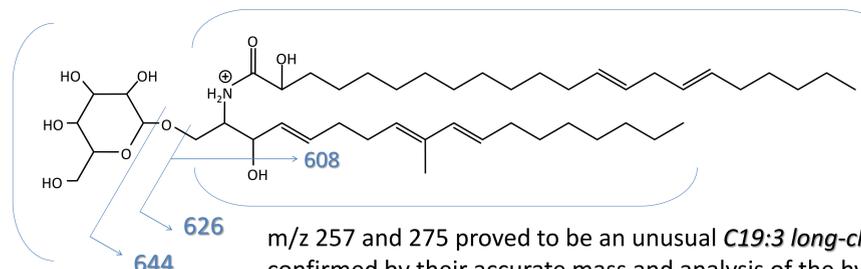
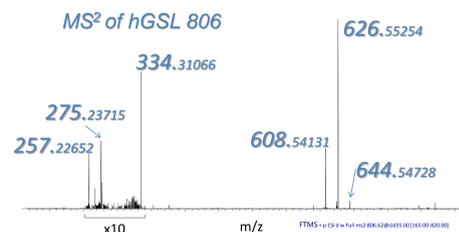
Before ... hGSL *host-glycosphingolipid*

hGSL is present whether the host is infected or not, and is found to have an unusual long-chain base.



All chromatograms are base peak plots, full scan 500-2000 m/z from the ion-trap MS. Internal std in grey.

The MS² spectrum of 806 shows a loss of 162 indicating a *sugar* headgroup attached to a *ceramide* core lipid. Further losses of 18 (H₂O) originate from the remaining hydroxyl groups.



hGSL
C₄₇H₈₄NO₉⁺
806.61406 Da calc
806.61337 Da obs

Accurate mass of product ion of m/z 334 observed in FT-MS data (not shown) indicates a *C22:2 hydroxy-fatty acid*.

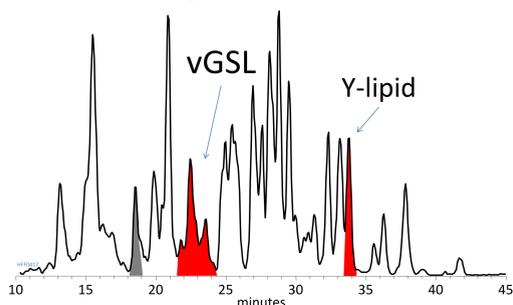
m/z 257 and 275 proved to be an unusual *C19:3 long-chain base (LCB)*, confirmed by their accurate mass and analysis of the hydrolysed LCBs. Interestingly, polyunsaturated LCBs do not produce the typical even-mass "LCB fragment" in MS² (as seen in sGSL). A C19:3 LCB is previously described (e.g.) in squid by Ohashi et al¹ and we have adopted their methyl-branched structure.

During ... vGSL *viral-glycosphingolipid* and Y-lipid

A group of enigmatic GSLs appear during infection, the long-chain bases of these vGSLs appear to have more than the usual number of -OH groups.

In addition, a glycerol-based polar lipid with a novel head group was observed to be *sequestering certain fatty acids* during infection, but did not change when not infected or in resistant strains.

Ehux374 + EhV86 @ 69 h



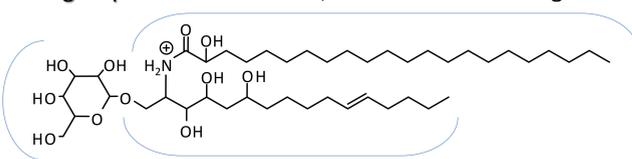
Other peaks in the chromatogram are mostly membrane lipids including glyco-, phospho- and betaine lipids.

MS² spectra of vGSLs show the same loss of 162; a *sugar* headgroup attached to a *ceramide*, but the MS² spectra are quite different due to differences on the long-chain base.

MS² of vGSL 804



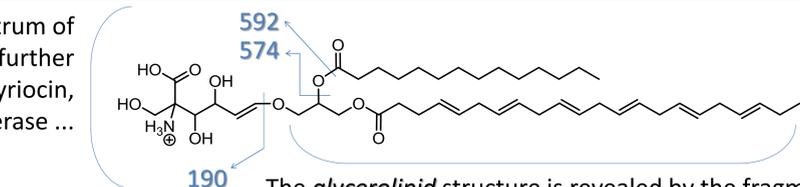
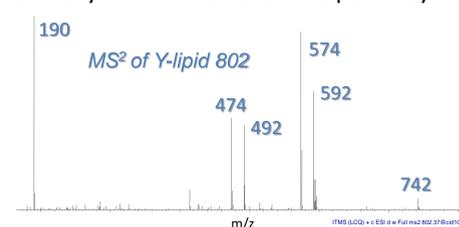
vGSLs were isolated and derivatized; each -OH takes on a trimethylsilyl moiety, an extra 72 Da. Analyzed by direct infusion, the derivatized mass of vGSL increased (e.g.) from 804 to 1380, indicating eight -OH groups on this molecule; four are from the sugar headgroup...



vGSL
C₄₄H₈₆NO₁₁⁺
804.61954 Da calc
804.65770 Da obs

Hydrolysis revealed a long-chain base that looks like a *C16:1 with four hydroxyl groups*, its MS² spectra compares favorably with phytosphingosine, a common long-chain base with 3 hydroxyl groups. Future high resolution mass spectrometry will aid this identification.

The characteristic headgroup ion seen in the MS² spectrum of Y-lipid at 190.07106 m/z corresponds to C₇H₁₂O₅N, further MSⁿ analysis suggests a structure like that of myriocin, coincidentally an inhibitor of serine palmitoyl transferase ...

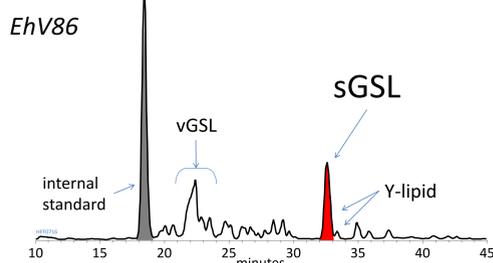


Y-lipid
C₄₆H₇₆NO₁₀⁺
802.54637 Da calc
802.54851 Da obs

The *glycerolipid* structure is revealed by the fragment pairs 592/574 and 492/474 in the MS² spectrum. These fragments result from the loss of one fatty acid and indicate *C14:0 and C22:6 fatty acids*. The C14:0/C22:6 version of Y-lipid is *only observed during infection*, uninfected or resistant strains contain C16:0, C18:1 along with C22:6, whilst the viral particles contained the C14:0/C22:6 and the C22:6/C22:6

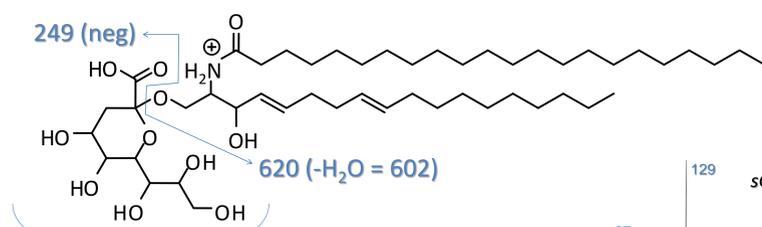
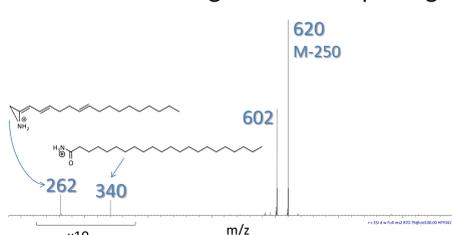
After ... sGSL *sialic acid-glycosphingolipid*

Purified by ultracentrifugation using a CsCl gradient, viral particles were found to be particularly enriched in vGSLs, Y-lipid and also sGSL, a glycosphingolipid with what appears to be a *single sialic acid headgroup*.



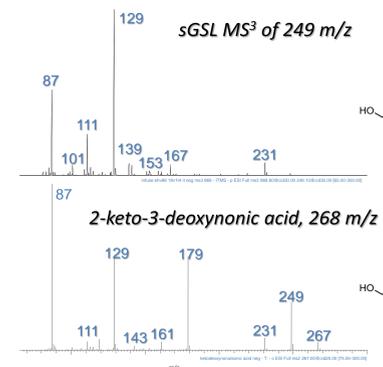
An internal standard was added to every sample before extraction.

The MS² spectrum of sGSL clearly shows the *d18:2/C22:0 ceramide core*, but the headgroup loss of 250 Da is larger than a simple sugar.



sGSL
C₄₉H₉₂NO₁₁⁺
870.66649 Da calc
870.66939 Da obs

Accurate mass of headgroup fragment 249.06160 observed in negative mode gives a formula of C₉H₁₃O₈⁻, further fragmentation of 249 gives a MS² spectrum (right, top) comparable to the MS² spectrum of the sialic acid *2-keto-3-deoxyonic acid* (right, bottom), which was obtained commercially and infused directly to obtain MS and MSⁿ spectra.



Abbreviations
HPLC (high performance liquid chromatography)
TMS (trimethylsilyl) BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide)
TMCS (trimethylchlorosilane) CsCl Cesium chloride

References
Ohashi et al, Journal of Lipid Research 41 2000 p1118-1124

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