Sphingolipid characterization of Volvocine algae reveals presence of glucosylceramides with α-hydroxylated 18:2 fatty acid and a possible role for sphingolipids in *Volvox carteri* embryo development

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Abstract:

Glucosylceramides (GlcCers) are sphingolipids composed of a long chain base (LCB), a fatty acid (FA), and a glucose head group. Previous studies in other model organisms have suggested GlcCers play an important role in multicellularity and cellular differentiation. In this study, we used the Volvocine algae, a collection of algae with unicellular and a variety of colony forming morphologies from the *Volvocales* order, to characterize GlcCers and other neutral sphingolipids in relation to colony morphology using HPLC-MS/MS.

We found Volvocine algal sphingolipids contain only saturated LCBs and synthesize GlcCers with a novel α-hydroxylated 18:2$^{\Delta 9,12}$ fatty acid containing ceramide. Total levels of GlcCers tended to increase in a slight trend with increasing complexity among the Volvocine algae with exceptions for *Pandorina morum* and *Volvox carteri*. Separation of gonidia and somatic cells from *Volvox* colonies followed by sphingolipid profiling revealed gonidia to have higher levels of ceramides and GlcCers, but lower hydroxyceramides relative to somatic cells. Additionally, gonidia sphingolipids appeared to have a higher proportion of long chain FA (16-18C) containing ceramides and hydroxyceramides whereas somatic cells had a higher proportion of very long chain FA (>18C) containing ceramides and hydroxyceramides.

We identified candidates for glucosylceramide synthase (GCS) DNA sequences from both *Chlamydomonas reinhardtii* and *Volvox carteri* genomes. The GCS sequence from *Chlamydomonas* contained two insertions of repeats, one in the predicted exon 4 and the other in the intronic region between exons 4 and 5 and did not appear to be expressed. However, the *Vovlvox* GCS was isolated from cDNA with a coding sequence of 1551 bp and complemented a *Δgcs1 Yarrowia lipolytica* strain when heterologously expressed.

Treatment of Volvocine cultures with the GCS inhibitor d,l-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) increased levels of ceramides in *Pandorina morum*, *Eudorina elegans*, *Pleodorina californica*, and *Volvox carteri*, and hydroxyceramides in *Gonium pectorale*, *Pandorina morum*, *Eudorina elegans*, and *Volvox carteri*. Only *Gonium* and *Pleodorina* showed significant decreases in GlcCers. The treatment had lethal effects for *Gonium* and *Volvox*, and repressed growth in *Pandorina* and *Eudorina*. *Chlamydomonas* exhibited no discernible changes in sphingolipid content or morphological changes under PDMP treatment. PDMP treatment of *Volvox* cultures resulted in embryos exhibiting a hollow, malformed shape as viewed under light microscopy. Together these results may suggest a role for sphingolipid metabolism in *Volvox* embryo development previously not explored.