BIODIVERSITY OF HIGHLY-GLYCOSYLATED PLANT PHOSPHOSPHINGOLIPIDS: FAST SCREENING AND STRUCTURE DETERMINATION BY TANDEM MASS SPECTROMETRY

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Glycosyl-Inositol-Phospho-Ceramides (GIPCs) are the main sphingolipids of plant tissues, but the function of this complex family of compounds remains largely unknown. Therefore, the structural characterization of GIPC represents a critical step toward the understanding of their physiological functions and their role in membrane organization. In this work, GIPCs have been purified from a wide variety of plants tissues or algae (23 species from various phylogenetic groups), yielding multiple molecular species that have been analyzed by tandem mass spectrometry, using a combination of MALDI- and ESI-MS/MS.

GIPCs were purified and extracted according to the method described by Buré *et al.*[1]. Once released from GIPCs, the quantitative distribution of fatty acid chains and long chain bases was analyzed by GC/MS[2]. GIPC extracts were analyzed by MALDI–MS (Ultraflex III, Bruker) in negative ion mode, using 2,6-dihydroxy-acetophenone (DHA) as a matrix. ESI-MS/MS analyses were performed with a 5500 QTRAP (AB Sciex) instrument.

Analyses performed by MALDI mass spectrometry provide a quick overview of the variety of GIPC structures found in a given type of plant tissue. MALDI-MS revealed that plant GIPC structures had an increasing number of saccharide units, from two (series A) to six (series E).

The 23 extracts obtained from plants belonging to various phylogenetic groups were analyzed first by MALDI-MS to attribute the different species to their corresponding series. ESI-MS/MS was carried out on these extracts to confirm or to elucidate some structures. Indeed, in algae, atypical GIPCs with several hexuronic acids were characterized by tandem mass spectrometry. In some other species, it appeared that inositol could be linked to one or two saccharides. The GIPC composition was revealed to be quite complex and different when analyzing extracts coming from primitive (algae) to more complex plants (dicot). We analyzed plant model organisms largely used by the scientific community like *Arabidopsis thaliana*, a model for reverse/forward genetic, or *Physcomitrella patens*, a moss used as a model organism for studies on plant evolution, development and physiology, but a real consensus for GIPC composition was not found. Thus, the analysis of the 23 extracts from primitive to more complex plants revealed a great diversity of GIPC structures in the plant kingdom, which is highly stimulating for further research in the plant sphingolipid field.

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[1] *C. Buré, J.L. Cacas, F. Wang, K. Gaudin, F. Domergue, S. Mongrand, J.M. Schmitter. Fast screening of highly glycosylated plant sphingolipids by tandem mass spectrometry. Rapid Commun. Mass Spectrom. 2011, 25, 3131.*

[2] *J.L. Cacas, S. Melser, F. Domergue, J. Joubès, B. Bourdenx, J.M. Schmitter, S. Mongrand. Rapid nanoscale quantitative analysis of plant sphingolipid long chain bases by GC-MS. Anal. Bioanal. Chem, 2012, in press.*