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Biochemical survey of the polar head of plant glycosylinositolphosphoceramides unravels broad diversity

Jean-Luc Cacas [a,1,2], Corinne Buré [b,2], Fabienne Furt [c], Jean-Paul Maalouf [d,e], Alain Badoc [f], Stéphanie Cluzet [f], Jean-Marie Schmitter [b], Elvire Antajan [g], Sébastien Mongrand [a,*]

[a] Université de Bordeaux, Laboratoire de Biogenèse Membranaire (LBM), UMR 5200 CNRS-Université Bordeaux Segalen, 71 Avenue Edouard Bourlaux, 33883 Villenave-d'Ornon Cedex, France
[b] Université de Bordeaux, Chimie Biologie des Membranes et Nanoobjets CBMN – UMR 5248 Centre de Génomique Fonctionnelle, Université Bordeaux Segalen, 146 rue Léo-Saignat, 33076 Bordeaux Cedex, France
[c] Worcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester, MA 01609, USA
[d] UMR BIOGECO INRA 1202, Ecologie des Communautés, Université Bordeaux 1, Bât. B2 RDC Est, Avenue des facultés, 33405 Talence, France
[e] UMR BIOGECO INRA 1202, INRA, 69 route d'Arcachon, FR-33612 Cestas Cedex, France
[f] Univ. de Bordeaux, ISVV, Groupe d'Etude des Substances Végétales à Activité Biologique (GESVAB), EA 3675, F-33140 Villenave-d'Ornon, France 3 avenue Espeleta, 33400 Talence, France
[g] IFREMER, 150 quai Gambetta, 62200 Boulogne-sur-Mer, France

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ABSTRACT

Although Glycosyl-Inositol-Phospho-Ceramides (GIPCs) are the main sphingolipids of plant tissues, they remain poorly characterized in term of structures. This lack of information, notably with regard to polar heads, currently hampers the understanding of GIPC functions in biological systems. This situation prompted us to undertake a large scale-analysis of plant GIPCs: 23 plant species chosen in various phylogenetic groups were surveyed for their total GIPC content. GIPCs were extracted and their polar heads were characterized by negative ion MALDI and ESI mass spectrometry. Our data shed light on an unexpected broad diversity of GIPC distributions within Plantae, and the occurrence of yet-unreported GIPC structures in green and red algae. In monocots, GIPCs with three saccharides were apparently found to be major, whereas a series with two saccharides was dominant in Eudicots within a few notable exceptions. In plant cell cultures, GIPC polar heads appeared to bear a higher number of glycan units than in the tissue from which they originate. Perspectives are discussed in term of GIPC metabolism diversity and function of these lipids.

1. Introduction

In animals, the main sphingolipid is represented by sphingomyelin, i.e. a ceramide molecule to which a phosphocholine head group is attached. By contrast to animals, plants and fungi do not possess such complex sphingolipids. Instead, they have inositol-containing glycosphingolipids known as Glycosyl-Inositol-Phospho-Ceramides, or GIPCs.

Among pioneering works on plant GIPCs is that of (Carter et al., 1958), which provided the first structural insights into the polar head and ceramide parts of the lipids (Carter et al., 1964; Carter and Kisic, 1969; Carter and Koob, 1969). The core structure of GIPCs consists of a ceramide moiety linked to an inositol–glucuronic acid unit via a phosphodiester bond (Cacas et al., 2012a). To this core structure, many diverse saccharides can be added, forming compounds like Gal-GlcR1-GlcA-inositol-1-phosphate-ceramide and GlcR1-GlcA-inositol-1-phosphate-ceramide in plants (where Gal refers to galactose, GlcR1 to glucose with a hydroxyl group, an amine or an acetylamine for R1, and GlcA to glucuronic acid). Additional saccharides found to be linked to inositol were arabinose, galactose, mannose and fucose in plants (Carter et al., 1969; Hsieh et al., 1981; Laine and Hsieh, 1987; for review Pata et al., 2010; Buré et al., 2013), and mannose, galactose, xylose, fucose, GlcN and GlcNAc in fungi (e.g. Loureiro y Penha et al., 2001; Heise et al., 2002; Gutierrez

Abbreviations: DHA, 2,6-dihydroxy-acetophenone; ESI, electrospray ionization; FA, fatty acid; GIPC, Glycosyl-Inositol-Phospho-Ceramide; Gal, galactose; GlcA, glucuronic acid; GlcN, glucosamine; Hex, hexose; IPC, inositol-phosphoceramide; hVLCFA, hydroxylated VLCFA; LCB, long-chain base; LCFA, long-chain fatty acid; MALDI-MS, matrix assisted laser desorption-ionization-mass spectrometry; MS/MS, tandem mass spectrometry; N-Ac, N-acetyl; Pen, pentose; VLFCA, very long-chain fatty acid.

* Corresponding author. Tel.: +33 05 57 12 25 37.
E-mail address: sebastien.mongrand@u-bordeaux2.fr (S. Mongrand).

[1] Present address: UMR 1347 Agroécologie INRA/uB/AgroSup, ERL CNRS6300, Pôle Interactions Plantes-Microroganismes, 17 rue Sully, 21000 Dijon, France.

[2] These authors contributed equally to this work, and must be considered as co-first authors.

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et al., 2007; Simenel et al., 2008). The number of saccharides linked to inositol is rather large in GIPC structures: up to 19 saccharides were potentially found in tobacco leaf GIPC (Kaul and Lester, 1975) and up to 20 saccharides in *Candida albicans* (Trinel et al., 2002).

More than 50 years later after GIPCs' discovery, little is still known about the structural diversity of these plant lipids. On the one hand, ceramide moieties of GIPCs have been extensively investigated, revealing specific long-chain base (LCB) and fatty acid (FA) profiles. LCB composition is dominated by tri-hydroxylated molecules such as monounsaturated and saturated phytosphingosine (t18:1 and t18:0, respectively) and FA content mostly consists of very long chain FA (VLCFA) with up to 26 carbon atoms, that are often hydroxylated in position 2 (hVLCFA; for review, see (Pata et al., 2010). On the other hand, structural data dealing with GIPC polar heads still remain scarce and keep hampering the elucidation of GIPC roles in membrane microdomain segregation and cell signalling in plants (Mongrand et al., 2010; Simon-Plas et al., 2011).

Because of their large hydrophilic polar head, solubilisation of GIPCs in usual organic solvents has been a major obstacle for their analyses. Indeed, most lipid extraction methods rely on phase partition in chloroform/methanol/water mixtures, but this is largely inefficient to extract GIPCs, that remain insoluble for the most part or are recovered in the aqueous phase and interphase Sperling et al., 2005; Markham et al., 2006). Recently, we have developed an extraction method adapted from (Kaul and Lester, 1975) to purify total plant GIPCs. Upon purification, GIPCs were solubilized in an acidic THF/methanol/water (4/4/1, v/v/v) solution and further characterized by MALDI-and ESI mass spectrometry. This optimized protocol provides structural information relative to the ceramide moiety and polar head of GIPCs, including the number and types of saccharide units (Buré et al., 2011).

Using this protocol, we have recently determined the GIPC composition of two plant models, i.e. *Arabidopsis thaliana* and *Nicotiana tabacum* (Buré et al., 2011). GIPCs were detected in a *m*/*z* 1200 to 2040 mass range, building six distinct series of compounds (hereafter referred to as series A to F, see Fig. 1). Within a series, GIPC molecular species, which share the same polar head, differed from one another by the ceramide moiety. Molecular mass differences recorded between species were 2, 14 and 16 Da, corresponding to differences in the number of unsaturation, carbon atoms and hydroxylation of the ceramide skeletons, respectively. Furthermore, mass spacing between series was found to be either of 162 or 132 Da, which corresponded to an additional hexose or pentose unit when compared to the previous series (Fig. 1). Thus, the identified GIPC series A possessed two saccharide units, series B three saccharide units and so on up to 7 saccharide units for series F. It is worth to note that all six GIPC series (A to F) were clearly observed in tobacco Bright Yellow-2 (BY-2) *in vitro* cell suspensions whereas series A was by far the most abundant in *Arabidopsis* foliar tissues and cell cultures (Buré et al., 2011). Series F was never observed in plant tissue, but was identified in BY-2 cell cultures (Buré et al., 2011).

Major tobacco and *A. thaliana* GIPCs were further characterized by tandem mass spectrometry. For instance, in the lowest mass range (*m*/*z* 1200 to 1340; series A), main compounds were identified as hexose(R1)-hexuronic acid-inositol-phosphoceramide. In *A. thaliana* leaves, the ceramide moiety was identified as t18:1 h24:0 with R1 being a hydroxyl group. Similarly, a h24:0 fatty acid chain was found in GIPCs from tobacco leaves, with an amine or an acetylamine for R1. In both *A. thaliana* and BY-2 cells, the presence of GIPC species differing from one another by 14 Da further suggested the occurrence of fatty acid chains with odd and even carbon numbers. This finding was confirmed by GC/MS analysis of fatty acids (Buré et al., 2011).

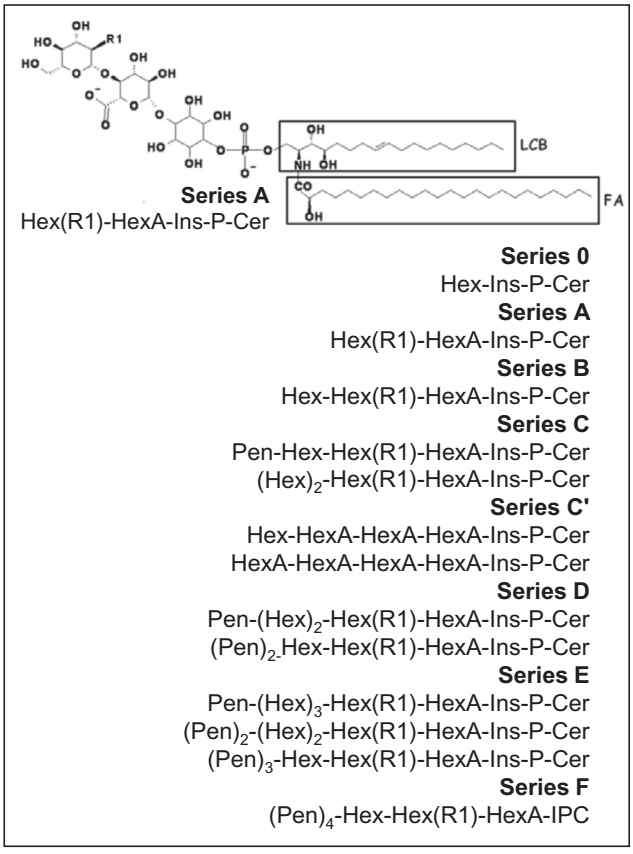

Fig. 1. Core structures of the different polar heads of plant GIPCs. *Top*, Typical GIPC of the series A found in most plants. R1 refers to OH, NH_2 or N(Ac): N-acetyl according to plant species (Pata et al., 2010); Bottom, Polar head variability of plant GIPCs. Seven GIPC series bearing from one (series 0) to seven saccharide units (series F) were detected in plant tissues. Abbreviations are as follow: Ins: Inositol; Cer: ceramide; FA: Fatty Acid; Gal: galactose; GlcA: glucuronic acid; GlcN: glucosamine; Hex: hexose; (hVLC)FA: (hydroxylated very long chain) fatty acid; LCB: Long Chain Base; Pen: pentose.

As aforementioned, little structural information is available in the literature regarding GIPC polar heads. This situation partly accounts for the lack of efficient ways to characterize GIPC structures. Having established a methodology to do so (Buré et al., 2011), we undertook a large scale-analysis of plant GIPCs in an attempt to tackle yet-unanswered questions. How diverse are GIPCs within Plantae? Does *A. thaliana* GIPC composition dominated by series A represent a rule in plants or a model peculiarity? Can GIPCs be found in algae, mosses and ferns? Are there any significant differences in GIPC polar head's between monocots and dicots? Do highly glycosylated GIPCs are more present in plant cell cultures than in tissues from which they originate?

In this work, 23 plant species were carefully chosen in diverse phylogenetic groups, from algae to angiosperms through Gnetophyta and gymnosperms. GIPCs were extracted, partially purified and their polar heads were characterized by mass spectrometry, as previously described (Buré et al., 2011).

2. Results and discussion

2.1. Choice of plant material

Twenty-three plant species ranging from algae to monocots and dicots were selected in different taxa in order to assess GIPC structural diversity within the plant kingdom. Whenever possible, plant model organisms used by the scientific community were chosen: *Fucus vesiculosus*, an algae used for embryo development investiga-