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GLYCOPHOSPHOSPHINGOLIPIDS: "GANGLIOSIDE-LIKE" GLYCOLIPIDS FROM PLANTS AND FUNGI

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INTRODUCTION

Glycophosphosphingolipids are inositol-containing membrane components whose chief distinguishing characteristic is a phosphodiester linkage between the sugar chain and the ceramide (1-16). These compounds, also unique in containing an inositol, have the following common core structure: Inositol(1→0)-phosphoryl(0→1)-ceramide, with higher homologs having extended sugar chains attached to the inositol (*ibid.*). Thus far, these substances have been found in plants, yeast and fungi, but not in any of the examined animal phyla. As negatively charged glycosphingolipids, these ubiquitous compounds in the plant kingdom may be analogous to, and may have as many structural varieties as, the sialic acid-containing compounds known as "ganglisides" found in animals.

Early work on glycophosphosphingolipids (GPS) was initiated by Professor H.E. Carter, then at the University of Illinois (1-3, 5-7). He and his co-workers reported that seeds from several crop plants, including cotton, peanut, corn, and soybeans, had glycolipids containing phytosphingosine, inositol and phosphate. Substances with similar chemical properties could be isolated from several plants, and were termed "phytoglycolipids." (Since it has now been reported that plants also contain glycosphingolipids which have sugars glycosidically attached directly to the ceramide (12,17), the term GPS is more useful to specify the phospho-inositol containing compounds.) Carter's work led to the structure of a tetrasaccharide from corn seeds reported as follows:

I. Man(α1→2)
GlcNAc(α1→4)GlcUA(α1→6) → Inos(1→0-phosphoryl)-ceramide

These workers found that upon carbon-celite and anion exchange chromatography the corn GPS fraction contained 41% of this tetrasaccharide, 9% of a trisaccharide (lacking the mannose), and 42% penta- and higher oligosaccharides. Carter's group also reported on GPS compounds from *Phaseolus vulgaris* (6) and on aminosugar-lacking GPS compounds from other plant seeds (7). High resolution chromatographic methods were not available to isolate the various species of each oligosaccharide fraction, and this pioneering group, having performed extensive chemical analysis of the available pure oligosaccharide, reported no further work on the subject.

During this time, a GPS compound in yeast was reported by Wagner and Zofcsik to have the following preliminary structure (4):

II. Mannose-Inositol-Phosphate-Ceramide (MIPC)

GPS compounds from peanuts were also investigated by Wagner (8), and a partial structure of one component was proposed as follows:

Ceramide-phosphoryl-Inos(μ→4)GlcUA(α1→3)GlcNH$_2$(1→μ)(Gal, Ara, Man)

This same group reported the isolation of a GPS fraction which had glucose and glucuronic acid from the alga *Scenedesmus obliquus* (9). This was the first report to include algae in the organisms which synthesize sphingosine.

In yeast and fungi, Lester, *et al.* (10-12), confirmed the presence of MIPC (II, above), found and characterized the following compound:

III. Inositol(1→0)-phosphoryl-ceramide (IPC)

They also isolated and partially characterized the following:

IV. (Inositol-phosphoryl)inositol-phosphoryl-ceramide (IP)$_2$C

V. (Inositol-phosphoryl)mannosyl-inositol-phosphoryl-ceramide M(IP)$_2$C

Kaul and Lester resumed research on plant GPS by development (14) of a mild extraction method for obtaining a GPS concentrate from fresh tobacco leaves. Carter's and Wagner's laboratories had used much harsher chemical extraction procedures (acid or alkali), which could have chemically altered the GPS compounds. Numerous bands were seen upon thin-layer chromatography of this GPS fraction and the two major compounds were found to have the following compositions: 1) $GlcNH_2$-GlcUA-Inos-Phos-Ceramide, and 2) its *N*-acetyl analog. In fact the total GPS fraction was nearly equally divided between compounds which had a free amino group on the glucosamine and compounds with an *N*-acetyl-glucosamine. In tobacco leaves, the trisaccharide constituted about 40% of the GPS fraction (whereas in corn, Carter had found only 9% trisaccharide). From the composition, it was possible to assign tentative sugar ratios for a series of 6 fractions which had, in addition to GlcNAc, GlcUA, and Inos, additional arabinose and galactose moieties (14).

The detailed structure of the tobacco trisaccharide was determined by Hsieh *et al.* (15) to be as follows:

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→ VI. GlcNAc*p*(α1→4)GlcUA*p*(α1→2)-Inos-(1→0-phosphoryl)-ceramide

This structure differed from the core structure of the compounds found in corn by having the glucuronic acid connected to the 2-position of the myoinositol, rather than the 6-position as reported by Carter *et al.* (5). Chemical ionization mass chromatography (18, 19) was used to detect the products of periodate oxidation to determine the position of linkage on the inositol (15).

A major tetrasaccharide from tobacco leaves

An advance in the chromatography of oligosaccharides was developed by Wells and Lester (20) utilizing reversed-phase HPLC of the reduced acetyl derivatives of malto-oligosaccharides as model compounds. This enabled the purification of some of the GPS oligosaccharides by repetitive chromatography on low-capacity reverse-phase columns (16). These inositol-containing oligosaccharides are non-reducing. Therefore the steps used to prepare the oligosaccharides for HPLC were 1) ammonolysis to release the phosphoryl-ceramide, 2) reduction of the glucuronic acid carboxyl (to prevent isomerization and decarboxylation) and 3) acetylation. The major tetrasaccharide obtained by this procedure had the following structure (16).

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→ VII. Gal*p*(β1→4)GlcNAc*p*(α1→4)GlcUA*p*(α1→2)Inositol

A minor tetrasaccharide, only partially purified, appeared to have the same composition as Carter's major tetrasaccharide from corn (5). The methylation linkage analysis of this compound indicated two terminal sugars, mannose and GlcNAc, and 4-substituted GlcUA, as well as disubstituted inositol. The anomeric configurations were all α as determined by CrO_3 oxidation (16).

According to methylation analysis (21), the next major member of this series appeared to have a galactopyranose(1→6)-linked to the terminal galactose of the major tetrasaccharide to give the following structure:

VIII. Gal*p*(1→6)Gal*p*(β1→4)GlcNAc*p*(1→4)GlcUA*p*(α1→2)Inositol

A minor component appeared on methylation analysis (21) to contain a terminal, arabinofuranoside(1→6)-linked to the terminal galactose of tetrasaccharide IV (described above) giving the following chain:

IX. Ara*f*(1→6)Gal*p*(β1→4)GlcNAc*p*(α1→4)GlcUA*p*(α1→2)Inositol

Both of these fractions appeared to be a mixture with one or more hexasaccharides, evidenced by the presence of 5-10 mol% of 2,4-di-O-methyl galactose which would indicate a 3,6-disubstituted galactose. About 5-10 mol% of terminal galactose was found in the pentasaccharide IX sample and a similar small amount of terminal arabinose in the pentasaccharide VIII sample. The branched hexasaccharides could originate from galactoses disubstituted with either or both arabinose and galactose. A tentative structure for these hexasaccharides (which co-chromatographed with the pentasaccharide fractions) is proposed as follows (21):

X. R(1→6) / R'(1→3) — Gal(β1→4)GlcNAc(α1→4)GlcUA(α1→2)Inos

R = Gal, Ara*f*

R' = Gal, Ara*f*

The separation and isolation of these compounds is still a challenging task, however, new methods for resolution of oligosaccharides in underivatized, reduced form on normal phase chromatography (22) show great promise. This may form a powerful combination with the already-mentioned chromatography of the acetyl derivatives by reverse-phase chromatography (21). Detection methods useful for these separations include derivatization of the amino group of the glucosamine with ^{3}H-acetate, chromogenic or fluorescent compounds.

DISCUSSION

It is evident, from Carter's pioneering work on the plant glycophosphosphingolipids, Wagner's efforts on yeast and algae, Lester's investigations on the yeast, fungi and tobacoo, and from our recent combined efforts to elucidate the structures of the tobacco GLS's, that there is a great complement of new and unusual glycolipid structures awaiting discovery in the non-animal eucaryotes.

The enigma of research in the animal glycolipids has been the lack of definition of absolute function for even one known structure, and the fact that so many complex, energetically expensive compounds and genetically expensive enzyme systems have evolved; . . . to what purpose?

Perhaps by realizing that the plants have evolved an analogous set of glycolipids, but of completely different sugar structure, we can design some innovative experiments to test for function. One of the most intellectually appealing possible roles proposed for cell-surface glycoconjugates is that they have some crucial recognition, sorting, or specific adhesion function for differentiating multicellular organisms. Certainly, having a set of known compounds to study is valuable, and we have this in the animal studies, but we are far from this knowledge in the plant and fungal kingdom. Still, the information that we have suggests that, the more complex the organism, the more complex are the cell surface components - including the glycolipids. A plausible kind of experiment would be to obtain mutants, missing some transferase for the terminal sugars, then the subterminal groups and so on. Next, an attempt to generate the whole organism from the mutated cells would be necessary, to begin an investigation of the consequences of loss of a certain structure or group of structures. This would be especially interesting and perhaps possible in plants, since these organisms often are capable of generating a whole plant from one cell isolated in tissue culture, whereas animal cells in culture have lost the ability to regenerate. It will be important, therefore, to characterize the plant and fungal GPS, on a taxonomic and evolutionary basis, to have a set of known compounds to compare in single-celled and multi-celled organisms; to compare with the animal analogs, and to begin experiments on function. Implicit in this idea is the necessity of developing quick and high resolution isolation and detection techniques for the GPS compounds, which seem to be the most difficult yet to study.

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