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Structural studies on the neutral glycosphingolipids of *Manduca sexta*

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Abstract

Glycosphingolipids (GSLs) have been implicated as playing major roles in cellular interactions and control of cell proliferation in muticellular organisms. Moreover GSLs and other sphingolipids such as sphingomyelins, ceramides and sphingosines serve a variety of roles in signal transduction. Hence, identification of structures of GSLs in different biota will shed light in understanding their physiological role. During this study, the major glycosphingolipid component present in the extracts of stage-12 and stage-17/18 metamorphosing adults of *Manduca sexta* was identified as mactosyl ceramide. We report the isolation of several ceramide disaccharides, a ceramide trisaccharide and a ceramide tetrasaccharide. The GSL structures were confirmed by high-resolution mass spectrometry and tandem mass spectrometry. The identity of the monosaccharides was proved using exoglycosidases. The predominant sphingosine chain-length varied from C-14 (tetradecasphing-4-enine) to C-16 (hexadecasphing-4-enine) in these GSLs. Sphingosines of both chain lengths were accompanied by their doubly unsaturated counterparts tetradecasphinga-4,6-diene and hexadecasphinga-4,6-diene. It is also interesting to note the presence of tetradecasphinganine and hexadecasphinganine in minute amounts in the form of a GSL in the extracts of *M. sexta*. The varying degrees of unsaturation in the sphingosine moiety of GSLs in *M. sexta* may be biologically significant in insect metamorphosis. The ceramide trisaccharides and ceramide tetrasaccharide belong to the arthro-series, The observation of fucose in the *M. sexta* GSLs is the first report of the presence of fucose in an arthroseries GSL.

Keywords: Glycosphingolipid; *Manduca sexta*; Sphingadiene; Tandem mass spectrometry

1. Introduction

Glycosphingolipids (GSLs) and other related sphingolipids such as sphingomyelins, ceramides and sphingosines are minor cell membrane constituents which play a major role in different biological effects such as regulating cell proliferation, migration, cytoskeletal organization and differentiation [1]. GSLs are ubiquitous in nature, having been isolated from prokaryotes, plants and animals [2,3], but efforts to examine insect GSLs have been limited to only a very small number of insect species [4]. The GSLs isolated from insects differ significantly from vertebrate GSLs in their carbohydrate moieties (Fig. 1). In vertebrates, the core disaccharide is lactose (β−Gal-(1→4)-Glc), with variations in the third and subsequent saccharide moieties leading to an array of GSLs, whereas in the invertebrates, lactose is replaced by mactose (β–Man-(1→4)-Glc) [3]. The presence of mannose in insect GSLs has been observed in cells of the mosquito *Aedes aegypti* [5], in larvae of the green-bottle fly *Lucilia Caesar* [6], in pupae of the blowfly *Calliphora vicina* [7], and in the embryo of *Drosophila melanogaster* [8]. It is interesting to note that the first example of α-(1→4) linked *N*-acetylgalactosamine in nature was found in the *L. caesar* [9]. The occurrence of *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) in GSLs is reported in *L.* caesar [5], *C. vicina* [10] and *D. melanogaster* [7].

GSLs are broadly classified as neutral, acidic or zwitterionic GSLs [11]. Members of the electrically neutral class of zwitterionic GSLs have been isolated from insects where a

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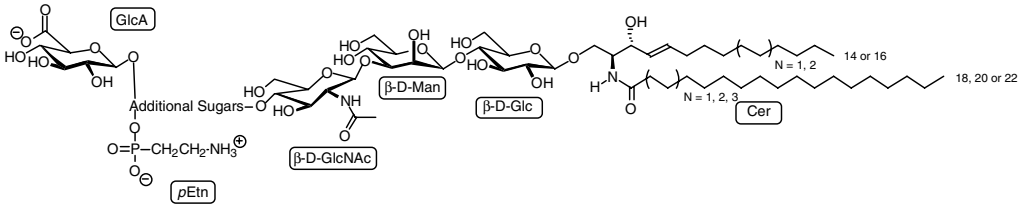

Fig. 1. Generalized structure of insect GSLs (arthrosides).

novel phosphonate group, phosphoethanolamine (*p*Etn), is tethered to one of the sugars in the carbohydrate chain of the GSL. The presence of phosphoethanolamine bound to *N*-acetylglucosamine has been observed in *L. Caesar* [10,12], *C. vicina* [13,14] and *D. melanogaster* [7]. Neutral GSLs with linear polysaccharides from *L. caesar* having chain lengths of eight or nine sugars [15] also have been reported. The acidic GSLs in insects are termed "arthrosides". Glucuronic acid (GlcA) has been observed in *L. Caesar* [16], *C. vicina* and *D. melanogaster* [7].

The insect ceramide (Cer)[1] moieties also vary from those found in vertebrates, with sphingosine chain lengths being shorter than those of vertebrates. The most frequently identified sphingosine bases in insect GSLs are $C_{14:1}$ and $C_{16:1}$. The major fatty acids have an even number of carbon atoms: C_{18}, C_{20}, and C_{22}. The presence of sphingadienes (tetradecasphing-4,6-dienine or doubly unsaturated sphingosine) in the ceramides, sphingomyelins and ceramide-phosphoethanolamines from *Manduca sexta* have been reported by Abeytunga et al. [17] in an earlier study.

To date the only study of the distribution of GSLs in different organs of an insect has been performed on *C. vicina* [18]. The highest levels of neutral GSLs were observed in the fat body. Higher amounts of zwitterionic GSLs were observed in the central nervous system and imaginal discs than in other tissues, and large amounts of acidic glycolipids were found in the imaginal discs.

In the current study, we sought to characterize the GSLs in the moth (Lepidoptera), *M. sexta*, a model system in which the structural and functional organization of numerous tissues such as muscle [19], eye [20], brain [21] and hormonal control [22] during metamorphosis; reproduction [23,24] have been studied extensively.

2. Materials and methods

2.1. Materials

DEAE-Sephadex A-25 (Cat. No. 17-0170-01) was purchased from Amersham Biosciences, USA. Dialysis tubing was spectra Por regenerated cellulose tubing, MWCO 6000–8000 Da and purchased from VWR (Cat. No. 25218-060). The dialysis tubing was kept for 20 min. in water before use to remove dry glycerin. Iatrobeads 6RS-8060 with particle size 10 μm and pore size 80 Å, and Iatrobead HPLC column 6RSP-8010-250, 4.6×250 mm were obtained from Shell-USA.

2.2. Generation of mass spectra

Electrospray ionization (ESI)-mass spectra were obtained using a Finnigan Ion-Trap (IT) LCQ classic HPLC/MS instrument in the positive ion mode. Typical ESI conditions were as follows: needle voltage 4.5 kV, capillary voltage 20 V, capillary temperature 200 °C, flow rate with infusion at 8 μl/min. The tandem mass spectrometry measurements (MS^n, $n = 2$–3) were performed in the ion trap (IT) with helium as a collision gas at a pressure of ca. 10^{-5} torr. Samples were electrosprayed in a $CHCl_3$:MeOH (1:4) solvent mixture. An IonSpec 4.7T Fourier transform ion-cyclotron resonance (FT-ICR) instrument equipped with an electrospray ionization (ESI) source was used to achieve accurate mass measurements with ultra-high mass resolution.

2.3. Generation of DEAE-Sephadex

DEAE-Sephadex A-25 (20 g) was swelled in 400 ml of water for 1 day. This was washed with 200 ml of $CHCl_3$:MeOH:0.8M NaOAc (30:60:8) for 4 times. Sephadex was left for 2 days at 4 °C in the final wash. Finally it was washed 4 times with 200 ml of $CHCl_3$:MeOH:H_2O (30:60:8). An aliquot of the final washing was evaporated on a watch glass to check for complete salt removal.

2.4. Extraction of GSLs from stage 17–18 metamorphosing adult animals

Animals were obtained from the A.R.L. Division of Neurobiology Insect Rearing Facility and were staged by observation of features visible through the cuticle with a fiber-optic light source (stage 12) and by examination of wing-pigmentation patterns (stage 17/18). Animals ($n = 80$) were chilled on ice for 20 min to anesthetize them and then pureed in a blender with 800 ml of acetone; the resulting material was filtered and the filtrate was saved. After a second acetone wash, the residue was further homogenized in 800 ml of $CHCl_3$:MeOH (1:2) and stirred at room temperature for

[1] *Abbreviations:* Cer, ceramide; CID, collision-induced dissociation; ESI, electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; Fuc, fucose; GlcNAc, *N*-acetylglucosamine; HPLC, high performance liquid chromatography; Man, mannose; MS/MS, tandem mass spectrometry; MS^n, *n*th order tandem mass spectrometry; TLC, thin-layer chromatography.

one day. After filtration the filtrate was saved and the residue was homogenized in 900 ml of $CHCl_3$:MeOH (1:1), and stirred at RT for one day. The residue so obtained was re-extracted in $CHCl_3$:MeOH (2:1) for one day. All $CHCl_3$:MeOH extracts were combined and the solvent was removed by rotary evaporation to yield 10.7 g of crude lipid extract. Saponification of these lipids were performed using 50 ml of 1 N methanolic KOH (1:1 methanol:water) at 40–45 °C for 6 h. After neutralization with 1 N HCl, the milky liquid was dialyzed against tap water for 3 days. Water was changed every 8 h. Freeze-drying the content of the tubing yielded 8.3 g of white material. This was re-dissolved in 50 ml of $CHCl_3$:MeOH:H_2O (30:60:10) and centrifuged to remove the insoluble material. After 3 extractions, the extracts were combined and passed over a DEAE-Sephadex column (10 g). Neutral GSLs were eluted using 150 ml of the same solvent $CHCl_3$:MeOH:H_2O (30:60:10). Rotary evaporation of the solvents gave 5.8 g of a crude neutral GSL fraction. The crude fraction was fractionated by HPLC on a 6×10 cm Iatrobeads column packed in $CHCl_3$:MeOH (8:2). The sample was loaded in $CHCl_3$:MeOH (8:2) and eluted with 150 ml of the same solvent. The column was further eluted with 150 ml of each of $CHCl_3$:MeOH (7:3), $CHCl_3$:MeOH (6:4), $CHCl_3$:MeOH:H_2O (120:70:17), $CHCl_3$:MeOH:H_2O (30:60:10). Fractions were collected in 25 ml test tubes. GSLs were isolated from the $CHCl_3$:MeOH:H_2O (120:70:17) fraction after checking the fractions using thin-layer chromatography (TLC). TLCs were visualized using orcinol in sulfuric acid. Sphingomyelin and ceramide phosphoethanolamine were also present in these fractions and these column fractions were further purified on HPLC according to the literature [25]. Stage-12 animals were also used and similar extraction protocol was followed separately for those animals.

2.5. Deacylation of mactosyl ceramide

Hydrolysis of the fatty acid chain was performed using the sphingolipid ceramide *N*-deacylase from *Pseudomonas* (Sigma Cata. No. S 2563). The reaction was done at 40 °C for 18 h in pH 5.5 acetate (0.02 M) buffer. Triton (0.8%) was added to the medium to solubilize the GSLs. Products were separated on a C-18 cartridges (Isolute SPE Part No. 221-0100-C) equilibrated in water. Compounds were eluted first with water, then with MeOH and finally with $CHCl_3$:MeOH (2:1). Lyso-sphingolipids were isolated in the $CHCl_3$:MeOH (2:1) fraction.

2.6. Exoglycosidase assays

Prior to the addition of the enzymes, the GSLs were mixed with the sodium salt of taurodeoxycholic acid and sonicated in the corresponding buffer for a few minutes. The following exoglycosidases were purchased for the enzyme assays: β-mannosidase from snail acetone powder, ammonium sulfate suspension (Sigma Cat. No. M 9400), *N*-acetylglucosaminidase from jack beans, ammonium sulfate suspension (Sigma Cat. No. A 2264) and α-L-Fucosidase from bovine kidney, ammonium sulfate suspension (Sigma Cat. No. F 5884). Mannosidase and *N*-acetylglucosaminidase treatments were done in pH 4.4 citrate buffer; fucosidase treatment was done in pH 4.8 citrate buffer. All reactions were done at 40 °C for 18 h. After the reaction, resultant GSLs were extracted to $CHCl_3$:MeOH (2:1) solution. The lower organic-phase was dried with K_2CO_3, filtered through cotton, and the solvent was removed. The residue was re-dissolved in $CHCl_3$:MeOH:H_2O (120:70:17) and spotted on Whatman K5F silica gel 150 Å (Cat. No. 4851-320). After running the TLCs in $CHCl_3$:MeOH:H_2O (120:70:17), the plate was developed with orcinol (0.2% orcinol in 1:4 H_2SO_4:H_2O).

3. Results

Several mactosyl ceramides (Fig. 2, Compound 1) were isolated from stage-17–18 metamorphosing adult *M. sexta*.

The identities of these GSLs were confirmed by using high-resolution mass spectra and tandem mass spectra. The chemical formulae were proven by high-resolution mass spectra (Table 1) obtained using FT-ICR spectrometer. The $[M+Na]^+$ peaks of the parent mactosyl ceramides were observed at *m*/*z* 856, 884, 912 and 940 (Fig. 3). The most abundant ions were observed at *m*/*z* 884 and 912. It was interesting to note the presence of a fully saturated mactosyl ceramides at *m*/*z* 858. Table 1 shows that the fragmentation pattern of this saturated ceramide is similar to that of normal mono-unsaturated GSLs. The peaks at *m*/*z* 884 and 912 were also accompanied by smaller peaks at *m*/*z* 882 and 910, but it was difficult to generate collision-induced mass spectra due to the low abundance of these ions.

In the tandem mass spectra, all of these mactosyl ceramides showed clear loss of the two hexoses, but further fragmentation to obtain ceramide-moiety chain length information was not successful as the daughter ion intensity was low. Hence, sphingolipid ceramide *N*-deacylase (Fig. 2) was used to enzymatically cleave the fatty acid moiety of the ceramides.

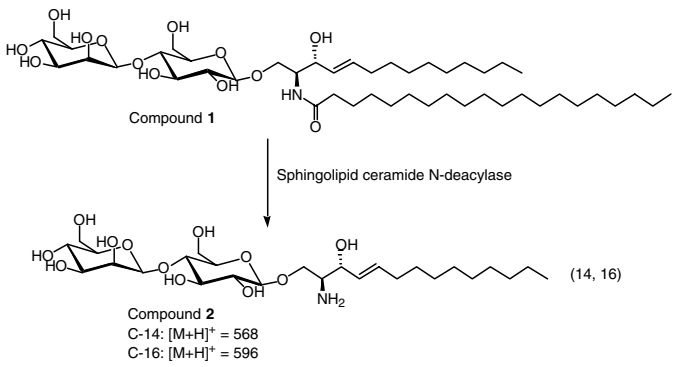

Fig. 2. Mactosyl ceramide (Compound 1) isolated from *Manduca*. Heterogeneity of the ceramide-sphingosine moiety was confirmed by enzymatic cleavage of the fatty acid.

Table 1
ESI MS and MS^n data of mactosyl ceramides isolated from stage-17–18 pupae of *Manduca*

Chain length (LCB/FA)	Molecular formula	$[M+Na]^+$ Calculated	Exact mass measured	$[MNa-162]^+$ (prominent)	$[MNa-162-162]^+$ (small)
d14:1/18:0	$C_{44}H_{83}NNaO_{13}^+$	856.5762	856.5766	694	532
d14:1/20:0	$C_{46}H_{87}NNaO_{13}^+$	884.6075	884.5938	722	560
d14:1/22:0	$C_{48}H_{91}NNaO_{13}^+$	912.6388	912.6282	750	588
d14:1/24:0	$C_{50}H_{95}NNaO_{13}^+$	940.6701	940.6710	778	—
14:0/18:0	$C_{44}H_{85}NNaO_{13}^+$	858.5919	858.5909	696	534

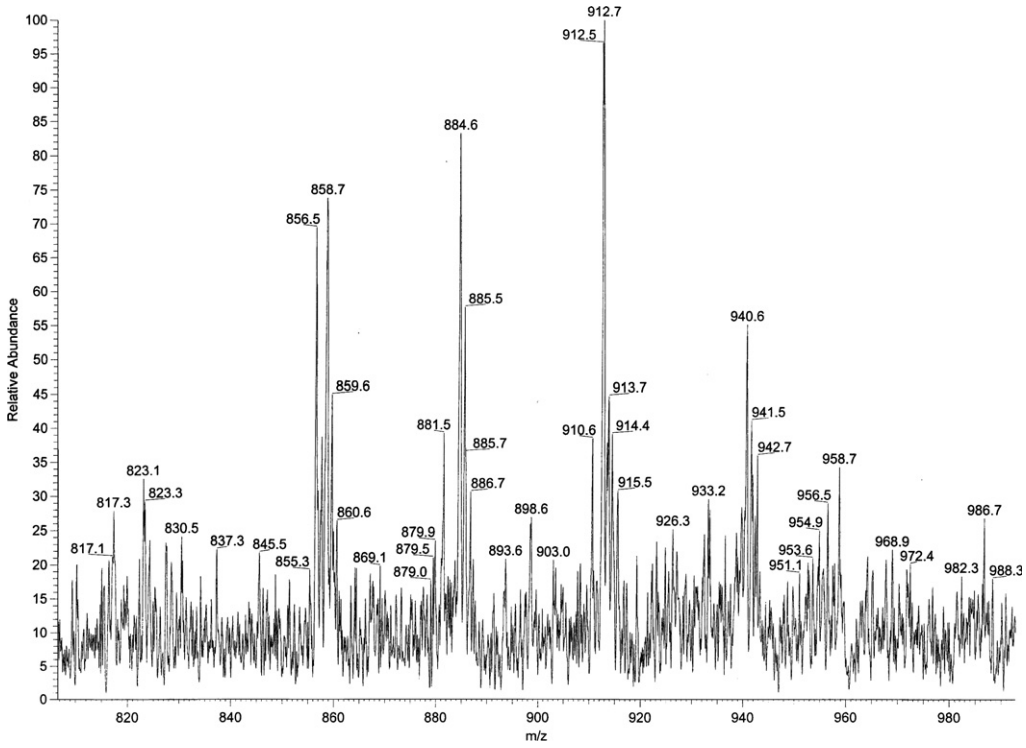

Fig. 3. The $[M+Na]^+$ peaks of the parent mactosyl ceramides are at *m/z* 856, 884, 912 and 940.

The lyso-sphingolipid (Compound 2) so obtained gave $[M+H]^+$ peaks in the mass spectra at *m/z* 568 and 596. The peak at *m/z* 568 was the most abundant. They were also accompanied by *m/z* 566 and 594. Trace amount of the fully saturated lyso-sphingolipid was also observed at *m/z* 570 and 598. ESI MS^2 data (Fig. 4) on parent-ion at *m/z* 568 showed the consecutive loss of two hexoses, finally giving the sphingosine fragment at *m/z* 208 indicating the major sphingosine chain length to be C-14. Data generated from the other $[M+H]^+$ are tabulated in Table 2. ESI MS2 data (Fig. 4) on parent-ion at *m/z* 596 gave the sphingosine fragment at *m/z* 236 showing the presence of C-16 sphingosine chain length as well.

The identity of the mannose was confirmed by β-mannosidase treatment, and this result agrees with earlier reports of β-mannose as the second monosaccharide in insect GSLs. This is the first observation, however, of an additional unsaturation in the ceramide, as well as the first observation of a fully unsaturated ceramide in an insect GSL.

In the same extract, the presence of hydroxyceramides was noted in the form of mactosyl ceramide at *m/z* 956.6619 (cal. 956.6650; $C_{50}H_{95}NNaO_{14}^+$). The corresponding fully saturated compound was observed at *m/z* 958.6794 (cal. 958.6807; $C_{50}H_{97}NNaO_{14}^+$). The corresponding GSL with sphingadiene was observed at *m/z* 954.6488 (cal. 954.6494; $C_{50}H_{93}NNaO_{14}^+$). ESI MS/MS data showed the loss of first hexose giving peaks at *m/z* 794, 796 and 792 respectively. The chain length information could not be generated using CID/MS due to the low abundance.

Ceramide trisaccharides were also observed in the extracts of stage-17/18 metamorphosing adult of *M. sexta*. The $[M+Na]^+$ peak of the parent GSL was observed at 1115.7235 (cal.1115.7182; $C_{56}H_{104}N_2NaO_{18}^+$). The ESI MS experiment showed a peak at *m/z* 912, indicating the loss of 203, which corresponds to a *N*-acetylated aminosugar. In the same mass spectrum the peak at *m/z* 750 indicated the loss of 162, corresponding to a loss of a second hexose. A structural homolog corresponding to the parent ceramide trisaccharide was also observed at *m/z* 1087. The monosaccharides were identified by exoglycosidase treatments; first with *N*-acetylglucosaminidase, and then by β-

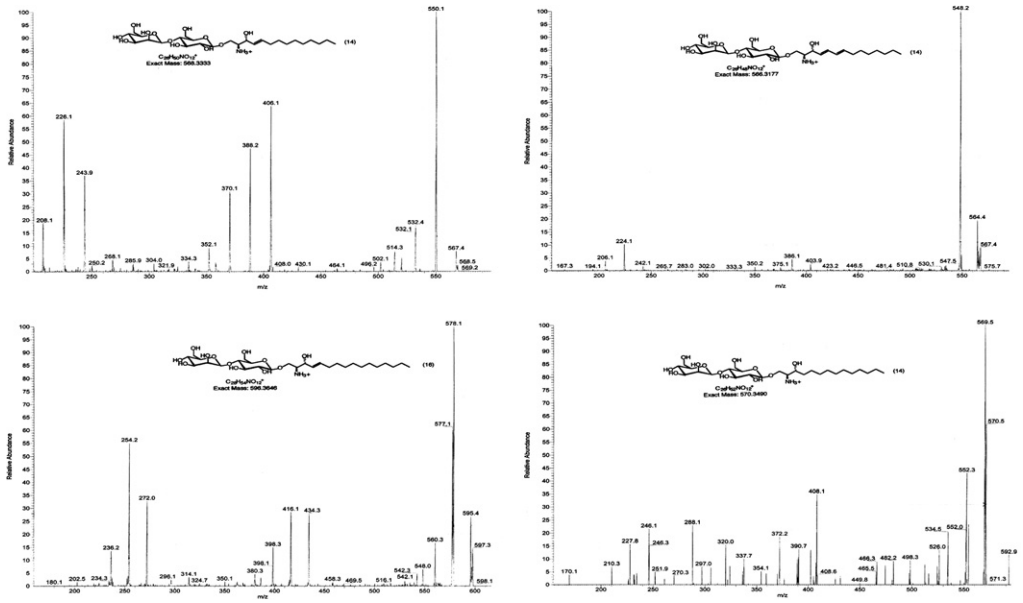

Fig. 4. ESI MS2 data on parent-ion lyso-sphingolipids at *m*/*z* 568, 596, 566 and 570. The lyso-sphingolipid at *m*/*z* 568 gave peaks at *m*/*z* 550 (loss of water), 406 (loss of a sugar), 244 (loss of two sugars). The peak at *m*/*z* 406 is accompanied by 388 (loss of water), 370 (loss of two water molecules). Simillarly loss of water molecules are observed from the peak at *m*/*z* 244 giving peaks at *m*/*z* 226 and 208.

Table 2
ESI MS^2 data of lyso-sphingolipid (Compound 2)

$[M+H]^+$	$[MH-162]^+$	$[MH-162-18]^+$	$[MH-(2\times162)]^+$	$[MH-(2\times162)-18]^+$	$[MH-(2\times162)-(2\times18)]^+$
566	404	386	242	224	206
568	406	388	244	226	208
570	408	390	246	228	210
594	432	414	270	252	234
596	434	416	272	254	236
598	436	418	274	256	—

Note that the data generated on *m*/*z* 566, 570, 594 and 598 are weak due to the low abundance of the parent ion.

mannosidase. Hence the ceramide trisaccharide could belong to the arthro-series structure such as GlcNAcβ1–3Manβ1–4Glcβ1Cer.

In addition, a ceramide tetrasaccharide was observed at *m*/*z* 1261. The high-resolution mass spectrum gave $[M+Na]^+$ at 1261.7800 (cal. 1261.7761; $C_{62}H_{114}N_2NaO_{22}^+$). The ESI MS/MS data showed a peak at *m*/z 1115, indicating the loss of 146 mass unit. The loss of 146 mass unit is indicative of a deoxy-hexose. The tandem mass experiments on the peak at *m*/*z* 1115 resulted in peaks at *m*/*z* 912 and *m*/*z* 750, indicating the loss of a *N*-acetylated amino-sugar and a hexose, respectively.

Once again, the identities of the monosaccharides were confirmed by exoglycosidase treatment. α-L-Fucosidase removed the fourth sugar, confirming the presence of a terminal α-L-fucose. β-*N*-acetylglucosaminidase and β-mannosidase cleaved the third and the second sugar, consecutively, confirming the presence of β-*N*-acetylglucosamine and β-mannose in the GSL. The R_fs of the resultant GSLs from the enzyme assays increased from ceramide tetrasaccharide to monosaccharide as expected with the mobile phase $CHCl_3$:MeOH:H_2O (120:70:17). However, the treatment of the ceramide tetrasaccharide with β-*N*-acetylglucosaminidase also cleaved the *N*-acetylglucosamine, indicating that the amino-sugar is also at a terminal position. This result suggests that the fucose forms an internal side-chain to the *N*-acetylglucosamine. The initial loss of fucose in the ESI MS/MS experiment can be explained by the more labile α-linkage of the fucose to the GSL. Thus, based on the MS and enzymatic cleavage data, the ceramide tetrasaccharide was inferred as being a fucosylated, arthro-series structure of GlcNAcβ1–3Manβ1–4(Fucα-2/3)Glcβ1Cer.

The same three types of GSLs were isolated in a similar fashion from stage-12 metamorphosing adults of *M. sexta*, suggesting that major GSLs are similar by the time the animals has completed roughly two-thirds of the period of adult metamorphosis.

4. Discussion

The major glycosphingolipid component present in the extracts of stage-12 and stage17/18 metamorphosing adults of *M. sexta* was identified as mactosyl ceramide. The length of the fatty acid moiety varied from C-18, C-20, C-22 to C-24. The predominant sphingosine chain-length varied from

C-14 (tetradecasphing-4-enine) to C-16 (hexadecasphing-4-enine). Both sphingosine chain lengths were accompanied by their doubly unsaturated counterparts tetradecasphinga-4,6-diene and hexadecasphinga-4,6-diene. It is also interesting to note the presence of tetradecasphinganine and hexadecasphinganine in minute amounts in the form of a GSL in the extracts of *M. sexta*. Sphinganines also have been observed in the ceramides of the metacestode, *Echinococcus multilocularis* [26], but the biological significance of the altered unsaturation levels of the sphingosine moiety in the ceramide of these invertebrate GSLs has yet to be determined. Sphingadienes similar to those reported in this investigation have been isolated from larvae of *Bombyx mori* [27] and from the marine annelid, *Neanthes diversicolar* [28].

It is well known [29] that the saturated nature of the GSLs causes denser packing and resulting in a higher melting temperature (T_m) of GSLs in comparison to the glycerolipids. The T_m is the temperature above which a bilayer of a single lipid-component switches from a solid-ordered (S_o) phase to a liquid-disordered (l_d) phase. In a model membrane containing mixtures of a high T_m lipid and cholesterol, a fluid-fluid phase separation has been observed [30] between the l_d and a liquid-ordered l_o phase. In addition, phase-behavior experiments using three different lipids of high T_m (di-saturated phosphatidylcholine or a sphingolipid), low T_m (mono-unsaturated phosphatidylcholine) and cholesterol have shown [31,32] that sterols have the ability to modulate the phase-separation of lipids. The co-existence of l_o, l_d, and S_o phases in ternary mixtures of sphingomyelin, mono-unsaturated phosphatidylcholine and cholesterol have been reported [33]. Hence it is worth investigating whether the presence of sphinganine, sphingosine and sphingadiene in the GSLs of *M. sexta* allows modulation of l_o, l_d, and S_o phases in the insect cell membrane due to the differences in the T_m of these membrane components caused by the differential packing. Because biologically active membrane proteins frequently occur as protein–lipid complexes [34], variation in the composition of GSLs with varying degrees of unsaturation may lead to the differential functionality of membrane proteins in *M. sexta*. Interestingly, sphingosine itself is also known to play a role in cell growth and viability by the inhibition of protein kinase C [35]. Thus the varying degrees of unsaturation in the sphingosines of *M. sexta* may permit different levels of protein kinase C inhibition, and in turn, modulate downstream signaling differentially during critical steps in insect metamorphosis.

The ceramide trisaccharide isolated from *M. sexta* was identified as GlcNAcβ1–3Manβ1–4Glcβ1Cer, which belongs to the arthro-series GSLs and is known to be expressed in both insects and nematodes [3]. It is interesting to note that in a recent study [36] of insect GSLs from a High Five cell line, Galβ3Manβ4Glcβ1Cer was identified as the main ceramide trisaccharide.

The ceramide tetrasaccharide from *M. sexta* was identified as GlcNAcβ1–3Manβ1–4(Fucα−2/3)Glcβ1Cer based on tandem mass spectrometry data and exoglycosidase cleavage experiments. The use of tandem mass spectrometry techniques in elucidating the structures of glycosphingolids [37] is considered to have considerable potential in deriving information regarding the structures of these minor cell components where the role of Nuclear Magnetic Resonance Spectroscopy is minor due to issues relating to the sensitivity.

The role of fucose in the GSLs of vertebrates has yet to be fully understood. It has been shown that nerve growth factor treatment of the pheochromocytoma cell line PC 12 enhanced fucosylglycolipid synthesis [38] and polyunsaturated, fucosylated GSLs have been found to be essential [39] for spermatogenesis and male mouse fertility. A fucosylated glycosphingolipid has been isolated from the millipede, *Parafontaria laminate* [40], and the structure was characterized as Manβ1–4(Fucα-3)Glcβ1-ceramide. Fucose has been also observed in the GSLs of the porcine parasite nematode, *Ascaris suum* [41]; the free-living nematode *Caenorhabditis elegans* [42]; in the GSLs of the human trematode parasite, *Schistosoma mansoni* [43] and in the GSLs of fresh-water bivalve molluscs [44,45]. Here in this study we now report its presence in the moth *M. sexta*. The role of *O*-fucosylation on proteins has been reviewed [46] and it is known that fucose on complex carbohydrates on cell surfaces play an important role in developmental processes. Although there are extensive reviews on glycosphingolipids present in various organisms [47] and their changes in health and diseases [48], the role of different sugars in the glycome of GSLs are largely unknown.

Moreover GSLs, sphingomyelins, ceramides and sphingosines serve a variety of roles in signal transduction [49,50]. The mechanism of sphingosine-1-phosphate signaling through its cell surface receptors and how this leads to different biological effects (regulating cell proliferation, migration, cytoskeletal organization and differentiation) [51] have been reviewed. Further it is also reported that sphingosine and lysosphingolipids are potent inhibitors of protein kinase C [52], an enzyme which plays a key role in signal transduction and cell regulation. Protein kinase C is known as a key element of the transduction routes that interact from induction to the end of the morphogenetic events during metamorphosis of a sea urchin [53]. In the present study, the presence of an extra unsaturation (or changes in the unsaturation) of the sphingosines in GSLs may have a physiologic function in regulating PKC activity during the metamorphosis of *M. sexta*. In conclusion, the role of sphingadiene(s) in the insect GSLs, ceramides, sphingomyelins has yet to be answered. Further studies are warranted in the synthesis of these spingadienes in order to better study the chemistry of these minor cell components in the living systems.

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