The Drosophila Gene Brainiac Encodes a Glycosyltransferase Putatively Involved in Glycosphingolipid Synthesis

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Summary

The *Drosophila* genes *fringe* and *brainiac* exhibit sequence similarities to glycosyltransferases. *Drosophila* and mammalian *fringe* homologs encode UDP-N-acetylglucosamine: Fucose-O-Ser β1,3-N-acetylglucosaminytransferases that modulate the function of Notch family receptors. The biological function of *brainiac* is less well understood. *Brainiac* is a member of a large homologous mammalian β3-glycosyltransferase family with diverse functions. Ten distinct mammalian homologs have been demonstrated to encode functional enzymes forming β1-3 glycosidic linkages with different UDP donor sugars and acceptor sugars. The putative mammalian homologs with highest sequence similarity to *brainiac* encode UDP-N-acetylglucosamine: β1,3-N-acetylglucosaminytransferases (β3GlcNAc-transferases), and in the present study we show that *brainiac* also encodes a β3GlcNAc-transferase that uses β-linked mannose as well as β-linked galactose as acceptor sugars. The inner disaccharide core structures of glycosphingolipids in mammals (Galβ1-4Glcβ1-Cer) and insects (Manβ1-4Glcβ1-Cer) are different. Both disaccharide glycolipids served as substrates for *brainiac*, but glycolipids of insect cells have so far only been found to be based on the GlcNAcβ1-3Manβ1-4Glcβ1-Cer core structure. Infection of High Five cells with baculovirus containing full coding *brainiac* cDNA markedly increased the ratio of GlcNAcβ1-3Manβ1-4Glcβ1-Cer glycolipids compared to Galβ1-4Manβ1-4Glcβ1-Cer found in wild type cells. We suggest that *brainiac* exerts its biological functions by regulating biosynthesis of glycosphingolipids.
Introduction

The neurogenic Drosophila gene brainiac plays essential roles in epithelial development in the embryo and in oogenesis (1;2). Brainiac shares sequence similarity with glycosyltransferases, together with another gene, fringe (3). Recently it was demonstrated that fringe encodes a genuine glycosyltransferase (4;5), raising the possibility that brainiac may also function as a glycosyltransferase enzyme. Fringe modulates functions of the Notch receptor by extending O-linked fucosylation sites in EGF modules of Notch. UDP-N-acetylgalactosamine: Fucα1-O-Ser β1,3-N-acetylgalactosaminyltrnasferases (O-Fuc β3GlcNAc-transferase) encoded by mammalian fringe orthologs control the O-linked fucosylation pathway and allow synthesis of the sialylated tetrasaccharide NeuAcβ2-3Galβ1-4GlcNAcβ1-3Fucα1-O-Ser (6). Fringe may compete with an alternate glycosylation pathway controlled by an UDP-Glucose: Fucα1-O-Ser β3glucosyltransferase (6).

In Drosophila, brainiac mutants produce defects that resemble those produced by loss of Notch function during oogenesis. Consequently, brainiac protein has been considered as a possible modulator of Notch activity (2). Brainiac activity is required in the developing germ line for proper organization of the follicle. Some of the defects associated with loss of brainiac activity in germ line cells resemble defects associated with loss of Notch activity. Recently, the role of Notch and its ligand Delta in signaling between germ line and somatic cells has been clarified (7). Delta is expressed in germ line cells and required for activation of Notch in somatic follicle cells at two stages of oogenesis. The

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1 The abbreviations used are: β3GlcNAc-transferase, UDP-N-acetylgalactosamine: acceptor β1,3-N-acetylgalactosaminyltrnasferase; Cer, ceramide; CDH, ceramide dihexoside; CTH, ceramide trihexoside; LacCer, lactosylceramide; MacCer, mactosylceramide; PCR, polymerase chain reaction; TOCSY, total correlation spectroscopy; gCOSY, gradient-enhanced correlation spectroscopy; NOESY, nuclear overhauser effect spectroscopy; gHSQC, gradient-enhanced heteronuclear single quantum correlation; gHMBC, gradient-enhanced
defects associated with loss of Notch activity resemble the defects associated with loss of brainiac in some respects, but differ in other respects. By analogy to the role of fringe as a modifier of Notch, it is possible that brainiac acts in the germ line to modify Delta and contribute to signaling between germ line and somatic cells. However, it is also possible that brainiac acts differently to influence multiple interactions between germ line and somatic cells.

In the present study we demonstrate that Drosophila brainiac is a β3GlcNAc-transferase similarly to fringe. However, brainiac has different acceptor substrate specificity and transfers to β-linked mannose as well as β-linked galactose residues. The core structure of Drosophila glycosphingolipids consists of mactosylceramide (Manβ1-4Glcβ1-Cer; MacCer), and this is extended by a β1-3 linked GlcNAc residue to the terminal mannose residue (8). It is suggested that brainiac has its primary role in glycosphingolipid biosynthesis and thereby affects glycosphingolipid mediated receptor modulation and functions mediated by lipid rafts.
**Experimental Procedures**

*Phylogenetic Analysis of the Drosophila and Mammalian β3Glycosyltransferase Family* – tBLASTn analysis with *brainiac* was used to search the *D. melanogaster* whole genome database GadFly released by the Berkeley Drosophila Genome Project (BDGP, Release 2 and 3) (9). Computed gene sequences were manually revised using EST cDNA information and the *Drosophila* Gene collection [DGC Release 1 and 2, (10)]. Amino acid sequences representing a central evolutionarily conserved domain of brainiac and orthologous *Homo sapiens* β3-glycosyltransferases were aligned with the identified *D. melanogaster* proteins using ClustalX 1.8 with Gonnet 250 protein weight matrix and default gap penalties (11). Multiple sequence alignments were revised for maximal residue conservation and minimal gaps in conserved amino acid sequence motifs. Distance analyses of the amino acid alignments were performed as described (12).

**Expression of Brainiac in Insect Cells** - An expression construct of the full coding region of *brainiac* was prepared by PCR using *D. melanogaster* (Canton S) genomic DNA (CLONTECH) and the sense primer MAB1 (5’-AGCGGATCCGCATGCAAAGTAAACACCGC-3’) and the anti-sense primer MAB3 (5’-AGCGGATCCTGCTACGCGTAATTGGCGG-3’) with *Bam*HI restriction sites. The PCR product was cloned into the *Bam*HI site of pVL1393 (PharMingen). Two N-terminal truncated constructs were prepared to obtain soluble secreted brainiac. One construct, pAcGP67-brainiac-sol, designed to encode amino acid residues 23-325 of brainiac was prepared by PCR using the primer pair MAB2 (5’-AGCGGATCCGACTATTGCGGCCTGCTGACC-3’) and MAB3 with *Bam*HI restriction sites. The PCR product was cloned into the *Bam*HI site of pVL1393 (PharMingen). A second construct, pAcGP67-brainiac-HIS-sol, designed to encode a N-terminal fusion of 6× His- and T7-tags to amino acid residues 28-325 of *brainiac* was prepared by PCR using the primer pair TSHC273 (5’-
CGAGGATCCGCTGACCCACCTGCACGAG-3') and MAB3 with BamHI restriction sites. The PCR product was fused to cDNA for six Histidine residues and a T7 tag interspaced by a thrombin proteolytic site, and the expression unit was cloned into the NotI restriction site of pAcGP67A (PharMingen). pVL1393-brainiac-full, pAcGP67-brainiac-sol, and pAcGP67-brainiac-HIS-sol were co-transfected with Baculo-Gold™ DNA (PharMingen) in Sf9 cells as described (13). Control constructs included pVL-fringe-myc and pVL-fringe-NNN-myc (4), where NNN represents an enzymatically inactive DxD motif mutant. Standard assays were performed in 50 μl total reaction mixtures containing 25 mM HEPES-KOH (pH 7.4), 10 mM MnCl₂, 0.1% n-octylglucoside, 100 μM UDP-[¹⁴C]-GlcNAc (2,300 cpm/nmol) (Amersham), and varying concentration of acceptor substrates (Fluka, Merck, Sigma and Toronto Research Chemicals Inc; see Table I for structures). For microsomal preparations High Five™ cells were lysed in 10 volumes hypotonic lysis buffer (25 mM HEPES-KOH (pH 7.4), 10 mM MnCl₂, 1% N-octylglucoside, 1 mM phenylmethylsulfonylefluoride), and membrane pellets obtained by differential centrifugation at 15,000 x g, followed by 150,000 x g were used at 150 mg/ml. The secreted brainiac constructs were assayed with 5-20 μl of culture supernatant from infected cells. The HIS and T7 tagged secreted brainiac protein was purified by Ni-NTA affinity chromatography (Qiagen) as described by the manufacturer. In addition this protein was immunoprecipitated with anti-T7 antibody agarose (Novagen) as described by the manufacturer, and enzyme assays were performed on washed beads as well as after elution at low pH. Reaction products of soluble acceptors were quantified by chromatography on Dowex 1-X8 (Sigma). Assays with glycosphingolipids included 5 mM 2-Acetamido-2-deoxy-D-Glucono-1,5-lactone (inhibitor of hexosaminidase activity), and products were purified on octadecyl-silica cartridges (Supelco) and analysed by thin-layer chromatography and autoradiography.

Isolation of CDH and CTH from High Five Insect Cells – High Five™ cells were grown in shaking upright roller bottles at 27 °C in serum-free medium (Invitrogen). Approximately 50 ml packed cells
were extracted in 2-propanol - n-hexane - water (55/25/20, v/v/v, upper phase removed) and subjected to Folch partition in chloroform - methanol - water (4/2/1, v/v/v). The dried lower phase glycolipids were freed from other lipids by peracetylation (pyridine-acetic anhydride 2:1 v/v), chromatography on Florisil, and base-catalyzed O-deacetylation (14). Further fractionation was carried out by preparative-scale HPLC. The structures of the di- and triglycosylceramide fractions were determined by $^1$H-NMR spectroscopy and electrospray ionisation mass spectrometry to be Manβ1-4Glcβ1-1Cer and Galβ1-4Manβ1-4Glcβ1-1Cer, respectively (M. Fuller, T. Schwientek, H. Clausen, and S.B. Levery, manuscript in preparation).

**Isolation of the Products Formed by Brainiac** – The products formed by Brainiac with Man-β-methylumbelliferone (Manβ1-MeUmb) (4 mg), High Five™ MacCer (2 mg) and human Galβ1-4Glcβ1-Cer (2 mg) were analyzed. The substrates were glycosylated with microsomes prepared from High Five cells infected with pVL-brainiac-full using thin-layer-chromatography to monitor reaction progress. The reaction products were purified by application to octadecyl-silica cartridges (Bakerbond, J.T. Baker), followed by stepwise elution with increasing concentrations of methanol in water (13); the product of reaction with MacCer was further purified by preparative-scale HPTLC (chloroform-methanol-0.5% aqueous CaCl$_2$, 50:40:10 v/v/v), with isolation of the fraction migrating as a trihexosylceramide, prior to NMR and MS analysis as described below.

$^1$H- and $^{13}$C-NMR Spectroscopy – Glycosphingolipid products were deuterium exchanged by repeated addition of CDCl$_3$-CD$_3$OD 2:1, sonication, and evaporation under nitrogen, then dissolved in 0.5 mL DMSO-d$_6$/2% D$_2$O (containing 0.03% tetramethylsilane as chemical shift reference) for NMR analysis. A 1-D $^1$H NMR spectrum was acquired on the product with LacCer on a 600 MHz Varian Inova spectrometer at 35°C, with solvent suppression by pre-saturation pulse. Its identity was established by comparison of the spectrum with those of relevant standards acquired under identical
conditions (15). For the product with MacCer, 1-D $^1$H, 2-D $^1$H-$^1$H gCOSY, TOCSY, and NOESY NMR spectra were acquired on a Varian Inova 800 MHz spectrometer at 55°C. The product with Manβ1-MeUmb eluting from the octadecyl-silica cartridge with 20%-30% MeOH was deuterium exchanged by repeated lyophilization from D$_2$O, and dissolved in 100% D$_2$O (containing a trace of acetone as chemical shift reference) for NMR analysis. 1-D $^1$H, 2-D $^1$H-$^1$H gCOSY and TOCSY, and 2-D $^1$H-detected $^1$H-$^{13}$C gHSQC and gHMBC NMR spectra were acquired on the Varian Inova 600 MHz spectrometer at 20°C; a directly detected 1-D $^{13}$C NMR spectrum was acquired on a Varian Inova 500 MHz spectrometer.

**MALDI-TOF Mass Spectrometry** – Molecular mass profiles of glycosphingolipids were acquired on an Axima-CFR (Shimadzu/Kratos Analytical, Manchester, England) MALDI-TOF mass spectrometer operating in positive ion reflectron mode (pulsed N$_2$ laser with delayed extraction; emission wavelength 337 nm; acceleration potential 5 kV). The matrix employed was 2,5-dihydroxybenzoic acid (DHB); samples were premixed with a solution of DHB (10 mg/mL) in acetonitrile-0.1% trifluoroacetic acid (1:1, v/v) prior to application and drying on the target. Molecular species were detected as their Na$^+$ adducts; angiotensin II and Pro$_{14}$Arg were used as external mass calibration standards (monoisotopic masses 1046.5 and 1533.9 D, respectively).

**Exoglycosidase Digestion** - Brainiac products formed with lactose, Galβ1-4Man and D-Mannose were prepared by incubating 1 μmol of acceptor sugars with 100 nmoles of UDP-[14C]-GlcNAc [3900 cpm/nmol] and Brainiac microsomes in reaction buffer. The reaction products were purified on Dowex 1-X8 and octadecyl-silica cartridges and freeze-dried. The resolubilized products were digested with 10U β-galactosidase (*E. coli*, Sigma) for 2 h or 312 mU β-N-acetylglcosaminidase (*Jack bean*, Sigma) overnight and purified by mixed bed resin chromatography (Sigma) followed by lyophilization.
Resolubilized samples were analyzed by thin-layer chromatography in chloroform-methanol-water (30:60:10 v/v/v) and autoradiography.
Results

Brainiac encodes an UDP-GlcNAc: βMan/βGal β1,3GlcNAc-transferase - Expression of the full coding region of brainiac resulted in marked increase in GlcNAc-transferase activity using free D-Mannose in a screen assay as developed for study of the activity of Fringe (4). Analysis of activities with monosaccharides at varying concentrations up to 500 mM with Brainiac and Fringe is shown in Figure 1. D-Mannose was the best substrate for Brainiac, but at high concentrations L-fucose and to a lesser extent D-galactose was used as acceptor as well. The activity of Brainiac with fucose was comparable with that of Fringe, and Fringe also appeared to have weak activity with mannose. It should be noted that the assays were quantitated for total microsomal protein but the relative levels of enzyme expressions are unknown, and the putative products with L-fucose were not characterized. The activities were only measurable at acceptor concentrations over 50 mM. In the case of fringe it did originally indicate that this enzyme functioned with O-linked fucose (4), and later studies suggest that mammalian fringe variants lunatic and manic exhibit distinct specificities for the peptide sequence carrying O-Fuc (16). The high activity at low concentrations brainiac exhibits with other substrates than fucose, clearly indicates that brainiac does not function in glycosylation of O-linked fucose. Table I summarizes activities obtained with a large panel of saccharide and saccharide derivative substrates using UDP-GlcNAc donor sugar nucleotide. No activity above background values was obtained with other donor sugar nucleotides (UDP-Glc, UDP-Gal, UDP-GalNAc, and UDP-Xyl) (not shown). Analysis of substrates containing terminal β-linked mannose (β-Man) and α-linked mannose showed strong preference for β-Man with monosaccharide derivatives and near exclusive activity for β-Man structures with disaccharides and larger. In agreement with free galactose serving as substrate several disaccharides with terminal β-Gal were used as acceptor. Galβ1-4Man, lactose and benzyl-β-lactose were substrates, while related N-acetylated structures (Galβ1-4ManNAc, N-acetyllactosamine, benzyl-
\(\beta\)-N-acetyllactosamine) were poorly active. Analysis of apparent \(K_m\) for the most active substrates identified showed that Man\(\beta\)1-MeUmb was the preferred acceptor substrate, and the disaccharides Gal\(\beta\)1-4Man and Gal\(\beta\)1-4Glc were used with significantly lower affinity (Table II).

Several attempts to generate a soluble secreted brainiac protein with good catalytic activity were performed. Initially, we used an untagged construct based on amino acid residues 23-325 but no activity was found in the culture medium of infected insect cells (not shown). Secondly, a \(N\)-terminally tagged construct based on amino acid residues 28-325 was expressed and a specific protein reactive with an anti-HIS antibody of predicted molecular weight was found by western blot SDS-PAGE analysis. Low activity with Man\(\beta\)1-MeUmb acceptor substrate was detected in the culture medium (approximately 2-3 fold over background). Purification of the HIS-tagged protein by Ni-NTA chromatography resulted in purification of an inactive protein (not shown). Immunoprecipitation with anti-T7 antibody resulted in specific precipitation of low activity (approximately 3-4 fold over background), but elution inactivated the protein. We did not further pursue the enzymatic properties of the soluble brainiac protein. In our experience several of the glycosyltransferases acting exclusively in the glycolipid biosynthetic pathways are enzymatically inactive as truncated soluble recombinant proteins expressed in insect cells (17;18).

Several parameters for the Brainiac assay with microsomal fractions were analysed for optimization. The most critical parameter was found to be the detergent solubilization. Triton X-100, Triton CF-54 and Nonidet P-40 had strong inhibiting effect on activity at 0.1 \%, while \(n\)-octylglucoside at 3.4 mM (0.1 \%) activated the enzyme. The pH optimum of brainiac activity was neutral (pH 7.4). Addition of 5 to 10 mM MnCl\(_2\) activated enzyme activity and MgCl\(_2\) and CaCl\(_2\) had no effect, while presence of EDTA destroyed the activity.
The product of Brainiac with Manβ1-MeUmb was determined by NMR analysis to be GlcNAcβ1-3Manβ1-MeUmb, as follows. As shown in Figure 2, panel A, the fraction of product eluted from octadecyl-silica by 20% MeOH exhibited sets of 1H resonances indicating a mixture of two compounds (two sets of β-Man H-1 and H-2 signals in proportion ~6:4, at 5.408/4.339 ppm and 5.436/4.242 ppm, respectively). One set clearly corresponds to unreacted starting material, and the other to a product of glycosylation by β-GlcNAc (additional H-1 signal at 4.757 ppm, 3J1,2 = 8.5 Hz, NAc signal at 2.085 ppm). Following complete assignment of 1H and 13C resonances from all three monosaccharide spin systems present (see Table III) by 2-D 1H-1H gCOSY and TOCSY, 1-D 13C and 2-D 1H-detected 1H-13C gHSQC experiments (not shown), the connectivity between the β-GlcNAc and the more abundant β-Man spin system was established unambiguously as a 1→3 linkage by a 2-D gHMBC experiment. This spectrum (Figure 2, Panel B) shows clear interglycosidic three-bond correlations between the β-GlcNAc H-1 and the downfield-shifted β-Man C-3 (79.61 versus 72.44 ppm), as well as between the corresponding β-HexNAc C-1 (98.81 ppm) and the downfield-shifted β-Man H-3 (3.986 versus 3.804 ppm). Slight upfield shifts of the corresponding β-Man C-2 and C-4 resonances in the product compared with the non-glycosylated starting material (Table III) are also consistent with the β1→3 linkage.

Brainiac showed high activity with the disaccharides Galβ1-4Man and Galβ1-4Glc. D-galactose and Galβ-MU were poor substrates with no activity detected at 20 mM. D-galactose in excess of 100 mM did, however, showed significant activity. Since the enzyme transfers GlcNAc to both D-galactose and D-mannose monosaccharides, it was necessary to determine which sugar served as the acceptor in the disaccharide substrate Galβ1-4Man. This was done by analysis of sensitivity to exoglycosidase treatment. The di- and trisaccharide products with lactose, Galβ1-4Man, and D-mannose were digested by β-N-acetylglucosaminidase and not by β-galactosidase treatment suggesting that the structures of
the brainiac products are GlcNAcβ1-3Galβ1-4Glc, GlcNAcβ1-3Galβ1-4Man, and GlcNAcβ1-3Man, respectively (Fig. 3).

**Brainiac functions in glycosphingolipid synthesis:** β-linked mannose is rare in eukaryotic glycoconjugates. The preformed dolichol-phosphate oligosaccharide precursor for N-glycosylation contains a Manβ1-4GlcNAc linkage, but this only serves as a substrate for α-mannosyltransferases. Brainiac was not active with hen egg albumin tested as previously described (19) indicating that high-mannose and hybrid type N-glycans do not serve as substrates (data not shown).

On the other hand, the core dihexosylceramide (CDH) of glycosphingolipids from flies (diptera), including D. melanogaster, and nematodes, including C. elegans, has been reported to be Manβ1-4Glcβ1-1Cer (MacCer), and this is extended by β1-3 linked GlcNAc in all structures characterized to date from these species (8;20-22). We therefore isolated MacCer as well as a major glycolipid migrating as a trihexosylceramide (CTH) from High Five insect cells. The latter was found to have the novel structure, Galβ1-4Manβ1-4Glcβ1-1Cer, instead of the GlcNAcβ1-3 terminated structure. As shown in Figure 4 brainiac uses both LacCer (lane 6) and MacCer (lane 7). The enzyme source used was a detergent solubilized microsomal fraction, and some endogenous products were produced (lane 5). These endogenous products appear to be genuine products of brainiac as control experiments with fringe microsomes did not produce these (lane 1). Two endogenous products migrating in the CTH region and one migrating in the ceramide tetrasaccharide region were observed. The identities of these remain unknown. Similarly, the endogenous product migrating in the ceramide tetrasaccharide region is likely to be GlcNAcβ1-3Galβ1-4Manβ1-4Glcβ1-1Cer based on the existence of Galβ1-4Manβ1-4Glcβ1-1Cer in High Five cells. The fastest migrating endogenous product almost co-migrated with the product formed with LacCer. This may suggest that LacCer is found in High Five cells in addition to the more predominant MacCer. Co-existence of MacCer and minor amounts of LacCer was previously
reported in *Calliphora vicina* (23). The product formed with LacCer migrated slightly faster than the product formed with MacCer.

A 1-D $^1$H NMR spectrum of the crude triglycosylceramide product formed with LacCer (not shown) exhibited resonances consistent with virtually complete conversion to GlcNAcβ1-3Galβ1-4Glcβ1-1Cer, i.e., anomeric signals at 4.620, 4.264, and 4.168 ppm ($^3J_{1,2} = 8.0$, 7.3, and 7.8 Hz, respectively), corresponding to H-1 of GlcNAcβ1-3, Galβ1-4, and Glcβ1-1 residues, as well as signals at 3.837 ppm, corresponding to H-4 of Galβ1-4 ($^3J_{3,4} = 2.6$ Hz), and at 1.836 ppm (singlet, 3H), corresponding to NAc of GlcNAcβ1-3, of this glycosphingolipid (compared with published values for these resonances in an NMR study of an authentic standard: 4.621, 4.265, 4.168 ppm [$^3J_{1,2} = 7.9$, 7.3, and 7.9 Hz, respectively]; 3.839 ppm [$^3J_{3,4} = 2.4$ Hz]; 1.837 ppm [singlet, 3H] (15)).

The triglycosylceramide product formed with MacCer was purified by preparative HPTLC (see Figure 5, lane 6), and confirmed by MALDI-TOF mass spectrometry and by 1-D $^1$H and 2-D $^1$H-$^1$H NMR spectroscopy to be authentic GlcNAcβ1-3Manβ1-4Glcβ1-1Cer (Ap3Cer). The major ions in the mass profile (Figure 6, Panel A) are consistent with Na$^+$ adducts of a glycosphingolipid product having the glycan formula HexNAc$\sim$Hex$_2$ attached to ceramides composed of d14:1 and d16:1 sphing-4-enines $N$-acylated with 18:0, 20:0, 22:0, and 24:0 fatty acids (predominantly d14:1/20:0 and d14:1/22:0, m/z 1087.5 and 1115.6, respectively), a profile reflecting the origin of the acceptor substrate, MacCer isolated from High Five cells (M. Fuller, T. Schwientek, H. Clausen, S.B. Levery, manuscript in preparation). NMR spectroscopic assignments for all $^1$H resonances in starting material and product, derived from high-resolution gCOSY and TOCSY experiments, are compiled in Table IV. As shown in Figure 7, the 1-D $^1$H-NMR spectrum of the product (Panel A), compared with that of the starting material (Panel B), exhibits an additional anomeric resonance, connected to a β-GlcNAc spin system
(H-1 at 4.539 ppm, $^3J_{1,2} \simeq 8$ Hz, overlapping the $\beta$-Man H-1 at 4.534 ppm). Although the near coincidence of the two H-1 signals in the product spectrum made it difficult to establish the linkage between the $\beta$-GlcNAc and $\beta$-Man by 2-D NOESY experiments (relevant correlation peaks were insufficiently resolved, even at 800 MHz; not shown), a comparison of the chemical shifts of all $^1$H resonances for $\beta$-Man in the starting material and product shows that the largest downfield glycosylation-induced shift change occurs for H-3 (3.470 versus 3.270 ppm; $\Delta\delta = 0.20$ ppm). Such a large downfield shift change is generally a reliable indication of the position of glycosylation, in the absence of unusual conformational effects or interactions between vicinally substituted residues, and in light of the linkage specificity already established for the Man$\beta$1-MeUmb product, the identity of the product with MacCer appears to be confirmed.

Figure 5 presents an HPTLC comparison of Folch lower phase GSLs extracted from High Five cells infected with a baculovirus expressing an irrelevant gene (lane 1) with those from High Five cells infected with baculovirus containing pVL1393-brainiac-full (lane 2). In the CTH region of High Five cells infected with an irrelevant virus (lane 1) the major band corresponds to Gal$\beta$1-4Man$\beta$1-4Glc$\beta$1-1Cer (Hi5-3). A small amount of GlcNAc$\beta$1-3Man$\beta$1-4Glc$\beta$1-1Cer (Ap3Cer), which migrates with a relative mobility slightly higher than Hi5-3 (compare migration of authentic standards analyzed under identical conditions, lanes 6 and 3, respectively), can also be observed in the wild type profile. This is consistent with results obtained previously (M. Fuller, T. Schwientek, H. Clausen, S.B. Levery, manuscript in preparation). In the CTH region of the wild type profile (lane 2), the amount of the higher Rf band is considerably greater, with intensity of staining almost equal to that of the Hi5-3 band. The identity of this higher Rf component was confirmed as follows. Total CTH fractions from control and brainiac infected High Five cells lower phase GSLs were isolated by preparative HPTLC; these fractions (Figure 5, lanes 4 and 5, respectively) were then analyzed by MALDI-TOF mass spectrometry under identical conditions (Figure 6, Panels B and C, respectively). The major ions in the
wild type CTH spectrum (Panel B) are consistent with Na\(^+\) adducts of a glycosphingolipid with the glycan formula Hex\(_3\)Cer, having a ceramide profile qualitatively and quantitatively similar to that already described above for the biosynthetic Ap\(_3\)Cer (again predominantly d14:1/20:0 and d14:1/22:0, \(m/z\) 1046.4 and 1074.5, respectively). The presence of a small amount of Ap\(_3\)Cer in the wild type profile is indicated by a set of less abundant Na\(^+\) adduct ions at \(m/z\) +41 increments (compare profile in Panel A). In the CTH spectrum from Brainiac-transfected cells (Panel C) the sets of Na\(^+\) adduct ions corresponding to HexNAc-Hex\(_2\)Cer and Hex\(_3\)Cer are almost equal in abundance, consistent with a substantial increase in the amount of Ap\(_3\)Cer relative to that of Gal\(\beta1\)-4Man\(\beta1\)-4Glc\(\beta1\)-1Cer, as observed by HPTLC analysis.
Discussion

The neurogenic gene brainiac was shown to encode a β3GlcNAc-transferase with broad acceptor substrate specificity having preference for β-Man but also showing significant activity with β-Gal terminating structures. Mannose linked β1-4 is found in the core structure of insect and nematode arthroseries glycolipids as mactosylceramide (8), and brainiac was shown to have activity with this glycolipid. Brainiac also showed activity with another disaccharide glycolipid, lactosylceramide, which represents the equivalent core structure upon which vertebrate glycosphingolipids are built. With the present knowledge of structures of Drosophila glycoconjugates mactosylceramide is the only likely natural substrate identified, indicating that brainiac serves an important function in the biosynthesis of glycosphingolipids.

The acceptor substrate specificity of Brainiac with various mono- and disaccharides and aglycon derivatives revealed clear preference for β-linked mannose (Table I). Furthermore, brainiac showed preference for dihexosides (Galβ1-4Glc and Galβ1-4Man), whereas disaccharides with penultimate N-acetylg glucosamine represented poor substrates (Galβ1-4GlcNAc and Manβ1-4GlcNAc). In agreement with this brainiac used MacCer and LacCer glycolipid substrates in in vitro tests. Extended glycosphingolipids of Drosophila and other dipterans have been reported to contain two β1-3 linked GlcNAc residues (e.g., Galβ1-3GalNAcβ1-4GlcNAcβ1-3Galβ1-3GalNAcα1-4GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-Cer) (20;24). Since brainiac showed poor activity with disaccharide structures containing internal n-acetylhexosamine and no activity with the disaccharide Galβ1-3GalNAcα1-benzyl it appears unlikely that brainiac also catalyses the addition of the outer GlcNAc residue (Table I).
Drosophila glycosphingolipids have all been reported so far to be based on the GlcNAcβ1-3Manβ1-4Glcβ1-1Cer (Ap3Cer) core structure and extended as discussed above. It is noteworthy though that relatively few studies have addressed Drosophila glycosphingolipids, as well as insect glycosphingolipids in general, compared to studies of vertebrate glycosphingolipids. In the biosynthesis of vertebrate glycosphingolipids built on LacCer, an important branch point occurs at the addition of the third monosaccharide residue. This is the determining step for synthesis of different classes of glycosphingolipids, designated (neo)lactoseries (GlcNAcβ1-3Galβ1-4Glcβ1-Cer), (iso)globoseries Galα1-3/4Galβ1-4Glcβ1-Cer), and ganglioseries (GalNAcβ1-4Galβ1-4Glcβ1-Cer) (25). These classes of glycolipids are differentially expressed in cell types and during cell differentiation (26-28) and have different properties and functions (25). There may be no analogous branch point in the biosynthesis of Drosophila glycosphingolipids, as only one CTH sequence, GlcNAcβ1-3MacCer, (Ap3Cer) has been reported from this species. On the other hand, it is possible that additional structures and pathways exist. Although Galβ1-4Manβ1-4Glcβ1-Cer was found as the major CTH component in High Five cells, and this structure may represent an aberrant pathway confined to cultured insect cells, its appearance implies that a Galβ1-4 transferase with substrate specificity for MacCer must be present in the insect repertoire. It is also possible that the alternative pathway relates to the lepidopteran, rather than dipteran, origin of the cell line. We were not successful in establishing a stable brainiac transfectant of High Five cells, but analysis of the glycosphingolipid profile of baculovirus infected cells showed that brainiac functioned and produced a significant shift in trihexoside ceramides to Ap3Cer. Brainiac is homologous to vertebrate β3GlcNAc-transferase enzymes that control the (neo)lactoseries pathway in vertebrates by forming GlcNAcβ1-3Galβ1-4Glcβ1-Cer (3;18;29-32), and brainiac was found to use LacCer similarly to the homologous mammalian
β3GlcNAc-transferases (Fig. 4). This provides strong support for the proposed role for brainiac in glycosphingolipid biosynthesis from a functional perspective.

The proposed function for brainiac in Drosophila glycosphingolipid biosynthesis implies that brainiac mutants may lack extended glycosphingolipids. Drosophila does not appear to have close brainiac homologs, which would be predicted to have similar functions (Fig. 8). More distant Drosophila homologs group independently or with vertebrate orthologs known to represent β3galactosyltransferases. It is therefore possible that this class of glycosphingolipids cannot be produced in brainiac mutant animals. Brainiac is required in the germ-line during oogenesis and is also expressed zygotically. At present it is not technically feasible to isolate sufficient numbers of maternally and zygotically mutant animals to permit analysis of the glycosphingolipid composition. Failure to extend glycosphingolipids beyond MacCer could also lead to lack of acidic and zwitterionic glycosphingolipids in Drosophila, which contain glucuronic acid linked to galactose residues and phosphoethanolamine linked to GlcNAc residues (8;24). Charged residues, glucuronic acid and sialic acids, of glycoconjugates are important for biological functions in vertebrates (33), and it is likely that glucuronic acid and phosphoethanolamine exert important functions in Drosophila as well.

The large vertebrate β3glycosyltransferase family homologous to brainiac (29) (Fig. 8) has been extensively characterized within the last few years. Functional subgroups with considerable apparent redundancies have been identified, and many of these have been assigned important roles in the biosynthesis of all glycosphingolipid classes in mammals. One group is represented by UDP-Gal: βGlcNAc β3galactosyltransferases, β3Gal-T1, -T2, and -T5, which are predicted to control synthesis of type 1 chain lactoseries structures on glycolipids and N- and O-linked glycoproteins (18;34;35). All three function in vitro with glycolipids, whereas only β3Gal-T2 has activity with N-linked glycoproteins, and only β3Gal-T5 functions with O-linked core 3 structures (29). Note that murine
β3Gal-T3 was originally erroneously proposed to function in lactoseries synthesis (36), however, β3Gal-T3, renamed as β3GalNAc-T1, is unique and functions in globo series glycolipid biosynthesis forming GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer (37). Surprisingly, a recent report indicated that this gene was essential in mice (38). However, the orthologous gene in man is inactivated in healthy individuals of the rare Pk blood group (39). β3Gal-T4 is also unique and functions in ganglioseries glycolipid biosynthesis forming Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-Cer (18;40). Again, β3Gal-T6 was originally erroneously reported as β3GnT with a β3GlcNAc-transferase activity similar to brainiac (41), however, a recent report shows that this gene encodes the Gal-I enzyme involved in the proteoglycan core region synthesis (Galβ1-3Galβ1-4Xylβ1-O-Ser) (42). A single Drosophila ortholog (CG8734) is predicted to have similar enzymatic functions. The human core 1 β3Gal-T (Galβ1-3GalNAcα1-O-Ser/Thr) is only distantly related and groups in an independent clade with two Drosophila orthologs (not shown) (43).

The dendrogram in Figure 8 based on protein distance analyses of the putative catalytic units of the β3glycosyltransferase family depicts brainiac in a subfamily with five mammalian orthologs, which are all known to function as β3GlcNAc-transferases. β3GnT2 functions in poly-N-acetyllactosamine synthesis (GlcNAcβ1-3Galβ1-4Glc[NAc]) of glycoproteins and glycolipids (30;44). β3GnT3 was shown to function as a core 1 extension enzyme (GlcNAcβ1-3Galβ1-3GalNAcα1-O-Ser/Thr) (45). The function of β3GnT4 may be related to the function of β3GnT2, although only low activity has been demonstrated thus far (30). β3GnT5 also has similar functions, and it may have a primary function in glycosphingolipid biosynthesis (GlcNAcβ1-3Galβ1-4Glcβ1-Cer) (31). Finally, the most distant of the close β3GlcNAc-T brainiac orthologs, β3GnT6, was recently shown to represent a core 3 enzyme (GlcNAcβ1-3GalNAcα1-O-Ser/Thr) (32). The mammalian β3GnTs thus all use βGal or
αGalNAc as acceptor sugar, while brainiac uses both terminal βGal and βMan. Human β3GnT2 in contrast to brainiac does not function with MacCer (T. Schwientek, unpublished).

The phenotypes associated with brainiac mutations have led to the proposal that it might modulate the activities of several signaling pathways, including Delta/Notch and TGFα/EGF (1;2;46). Could the diversity of effects observed in these mutants be due to an influence of glycosphingolipids on signaling? In vertebrates, glycosphingolipids are known to modulate receptor function and signaling pathways through direct interaction with receptors (47;48) and through formation of lipid rafts which provide structurally distinct membrane domains for localization of receptors and ligands (47;49). Drosophila lipid rafts have been identified and shown to be enriched with MacCer (50). It is therefore proposed that Brainiac exerts its biological functions in Drosophila by directing glycosphingolipid biosynthesis, rather than by directly modifying receptor molecules as established for Fringe. Brainiac mutants may therefore provide a unique genetically tractable system to study the biological role of glycosphingolipids in vivo.
References


Footnotes

For page 3:

1 The abbreviations used are: β3GlcNAc-transferase, UDP-N-acetylglucosamine: acceptor β1,3-N-acetylglucosaminyltransferase; Cer, ceramide; CDH, ceramide dihexoside; CTH, ceramide trihexoside; LacCer, lactosylceramide; MacCer, mactosylceramide; PCR, polymerase chain reaction; TOCSY, total correlation spectroscopy; gCOSY, gradient-enhanced correlation spectroscopy; NOESY, nuclear overhauser effect spectroscopy; gHSQC, gradient-enhanced heteronuclear single quantum correlation; gHMBC, gradient-enhanced heteronuclear multiple bond correlation; MeUmb, 4-methylumbelliferone; β3Gal-T, UDP-Galactose: acceptor β1,3-galactosyltransferase.

Acknowledgement

The authors wish to thank Heidi Geiser (Department of Chemistry, University of New Hampshire) for her expert help in acquisition of MALDI-TOF mass spectra, the University of Georgia Complex Carbohydrate Research Center for use of their Varian 500, 600, and 800 MHz NMR spectrometers, and Dr. John Glushka for providing invaluable assistance with NMR data acquisition.
Tables

Table I. Substrate specificities of Brainiac β1-3-N-acetylglucosaminyltransferase

Table II. Kinetic properties of Brainiac β1-3-N-acetylglucosaminyltransferase

Table III. $^1$H, $^{13}$C chemical shifts (ppm) and $^3J_{1,2}$ coupling constants (Hz, in parenthesis) for Manβ1-MeUmb substrate and biosynthetic GlcNAcβ1-3Manβ1-MeUmb product

Table IV. $^1$H chemical shifts (ppm) and $^3J_{1,2}$ coupling constants (Hz, in parenthesis) for Manβ1-4Glcβ1-1Cer substrate and biosynthetic GlcNAcβ1-3Manβ1-4Glcβ1-1Cer product
Figure Legends

Fig. 1. β3GlcNAc-transferase activities of Brainiac and Fringe with monosaccharides.

Microsomes of transfected High Five™ cells (3 mg protein) were used as enzyme sources. ●, Brainiac activity with D-mannose; ○, Fringe activity with D-mannose; ▣, Brainiac activity with L-fucose; □, Fringe activity with L-fucose; ◆, Brainiac activity with D-galactose; ◇, Fringe activity with D-galactose. Monosaccharides D-glucose, D-GalNAc, D-GlcNAc, and D-xylose are not included, as assays at 5 mM, 50 mM and 500 mM did not detect enzyme activity. Background values obtained with identically treated microsomal fractions expressing pVL-fringe-NNN-myc were subtracted.

Fig. 2. 600-MHz NMR spectra (100% D2O, 20°C) of product of Brainiac with Man-β-Methylumbelliferyl. Panel A, expansion of monosaccharide ring methine and hydroxymethyl proton region of 1-D 1H-NMR spectrum; Panel B, corresponding region of 2-D 1H-detected 1H-13C gHMBC spectrum. Interglycosidic three-bond correlations are marked by ovals in Panel B. MU, 4-methylumbelliferyl; S, resonances corresponding to starting material; P, resonances corresponding to product.

Fig. 3. Exoglycosidase digestion of brainiac products formed with Galβ1-4Man, Galβ1-4Glc and D-Mannose. Autoradiography of high performance thin layer chromatography of Brainiac products digested with Jack bean β-N-acetylglucosaminidase (lanes 1-6) and E. coli β-galactosidase (lanes 7-12). Chromatography of purified saccharides was performed in chloroform-methanol-water (30/60/10, v/v/v). The migration of standard mono- and
disaccharides is indicated. Complete digestion of disaccharide substrates by β-galactosidase was observed by orcinol-staining (not shown). β-GlcNAcase, β-N-acetylglucosaminidase.

Fig. 4  **Brainiac uses Mac-Cer and Lac-Cer glycosphingolipid substrates.** Assays were performed with microsomal fractions of High Five cells expressing full coding constructs of Fringe (*lanes 1-4*) and Brainiac (*lanes 5-8*). Autoradiography of high performance thin layer chromatography of reaction products (4 hours) purified by SepPak C-18 chromatography. Plate was run in chloroform-methanol-water (60/38/10, v/v/v). Migration of standard glycolipids is indicated. LacCer, lactosylceramide; MacCer, mactosylceramide; H5CTH, High Five™ ceramide trihexoside.

Fig. 5  **HPTLC analysis of glycosphingolipids from High Five cells infected with baculovirus expressing full coding brainiac.** Compared are total Folch lower phase glycosphingolipids from High Five cells infected with baculovirus expressing an irrelevant protein (*lane 1*); High Five cells infected with baculovirus expressing full coding brainiac (*lane 2*); authentic Galβ1-4Manβ1-4Glcβ1-1Cer (Hi5-3) isolated and previously characterized from High Five cells (*lane 3*); total CTH fraction from High Five cells infected with baculovirus expressing an irrelevant protein (*lane 4*); total CTH fraction from High Five cells with baculovirus expressing full coding brainiac (*lane 5*); GlcNAcβ1-3Manβ1-4Glcβ1-1Cer (Ap3Cer) produced by *in vitro* enzymatic glycosylation of High Five Manβ1-4Glcβ1-1Cer (MacCer) with Brainiac (*lane 6*). Solvent system was chloroform-methanol-0.5% aqueous CaCl₂ (50:40:10 v/v/v), and plates were developed with orcinol H₂SO₄ staining.
Fig. 6. **MALDI-TOF mass spectrometry of purified triglycosylceramide fractions from High Five cells infected with baculovirus expressing full coding brainiac.** Compared are sodiated molecular ion profiles of GlcNAcβ1-3Manβ1-4Glcβ1-1Cer produced by in vitro enzymatic glycosylation of Manβ1-4Glcβ1-1Cer with Brainiac (Panel A); total CTH fraction from wild type High Five cells (Panel B); total CTH fraction from Brainiac-transfected High Five cells (Panel C). Only monoisotopic peaks are labeled. Fractions analyzed by MALDI-TOF are the same as those analyzed by HPTLC shown in Figure 5 (corresponding to lanes 6, 4 and 5, respectively).

Fig. 7. **Downfield region of 800-MHz 1H-NMR spectrum (DMSO-d6/2% D2O, 55°C) of GlcNAcβ1-3Manβ1-4Glcβ1-1Cer produced by in vitro enzymatic glycosylation of Manβ1-4Glcβ1-1Cer with Brainiac (Panel A).** A spectrum of the starting material acquired under identical conditions is reproduced in Panel B. Arabic numerals refer to ring protons of residues designated by Roman numerals or capital letters in the corresponding structure. R refers to protons of the sphingosine backbone; cis refers to vinyl protons of unsaturated fatty-N-acyl components. Asterisks * denote resonances from other lipid impurities; two ** appear where large interfering peaks have been truncated for clarity.

Fig. 8. **Phylogram of β3-glycosyltransferases in Drosophila melanogaster and Homo sapiens.** The consensus tree from protein distance analyses of predicted catalytic β3-glycosyltransferase domains is based on progressive sequence alignments as described in “Experimental Procedures”. Bootstrap percentage values from 1000 replicates are indicated above the nodes. Putative D. melanogaster β3-glycosyltransferases are indicated by their GadFly annotation. β3GalT, β3GnT and β3GalNAcT indicate human β3-galactosyltransferases, β3-N-acetylglucosaminyltransferases, and β3-N-acetylgalactosaminyltransferases, respectively.
Phylogenetic subfamilies are indicated by alternate background shading. Sequence alignments included amino acids 78 to 315 of human β3GalT1 (GenBank accession E07739), 151 to 394 of β3GalT2 (GenBank accession Y15060), 78 to 320 of β3GalNAcT (β3GalT3, GenBank accession Y15062), 71 to 343 of β3GalT4 (GenBank accession Y15061), 56 to 296 of β3GalT5 (GenBank accession AB020337), 57 to 313 of β3GalT6 (GenBank accession AY050570), 142 to 387 of β3GnT2 (GenBank accession AB049584), 107 to 355 of β3GnT3 (GenBank accession AB049585), 118 to 360 of β3GnT4 (GenBank accession AB049586), 88 to 333 of β3GnT5 (GenBank accession AB045278), and 117 to 367 of β3GnT6 (GenBank accession AB073740). The analysis included amino acids 78 to 316 of *Drosophila melanogaster* brainiac (GenBank accession U41449), 85 to 342 of CG3038 (DGC accession AY061226), 109 to 359 of CG8976 (DGC accession AY071036), 99 to 366 of the predicted CG8734 protein sequence, 329 to 563 of the predicted CG8668 protein, and 134 to 387 of the predicted CG11357 protein. The predicted CG8673 cDNA sequence was manually revised as described (12) and found to encode a protein of 364 amino acids, amino acids 109 to 350 were included in the analysis.
<table>
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<td>Manα1-6Manα1-Me</td>
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<td>Manα1-6(Manα1-3)Manα1-Bzl</td>
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<td>Manα1-3(Manα1-6)Manα1-6(Manα1-3)Man</td>
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<sup>a</sup> Enzyme sources were microsomal preparations of pVL-<i>Brainiac</i> infected High Five™ cells (see “Experimental Procedures”). Background values obtained with microsomes of cells infected with an irrelevant construct were subtracted.

<sup>b</sup> Assayed at DMSO concentrations above 5% (v/v) due to limited substrate solubility. High DMSO concentrations inhibit Brainiac activity (not shown).

<sup>c</sup> Bzl, benzyl; Me, methyl; MeUmb, 4-methyl-umbelliferyl; Nph, nitrophenyl; ND, not determined.
Table II

Kinetic properties of Brainiac β1-3-N-acetylglucosaminyltransferase

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<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
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<tr>
<td></td>
<td>mM</td>
<td>nmol/h/mg</td>
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<tr>
<td>Manβ1-MeUmb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ± 0.7</td>
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<td>Galβ1-4Man&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>17.9 ± 0.6</td>
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<sup>a</sup> Enzyme sources were as described in Table I.

<sup>b</sup> Determined with donor substrate UDP-[<sup>14</sup>C]-GlcNAc (300 μM).

<sup>c</sup> Determined with acceptor substrate Galβ1-4Man (40 mM).
Table III

$^1$H, $^{13}$C chemical shifts (ppm) and $^3J_{1,2}$ coupling constants (Hz, in parenthesis) for Manβ1-MeUmb substrate and biosynthetic GlcNAcβ1-3Manβ1-MeUmb product

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$^a$ Data were obtained in D$_2$O at 20°C. Chemical shifts are referenced to internal acetone (set to 2.225 and 30.00 ppm for $^1$H and $^{13}$C, respectively).
Table IV

\(^1\)H chemical shifts (ppm) and \(^3\)J\(_{1,2}\) coupling constants (Hz, in parenthesis) for Man\(_{1-4}\)Glc\(_{1-1}\)Cer substrate and biosynthetic GlcNAc\(_{1-3}\)Man\(_{1-4}\)Glc\(_{1-1}\)Cer product

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<td>H-1</td>
<td>4.512 (~1) 4.158 (7.7) 3.464, 3.947</td>
<td>4.539 (~8) 4.534 (~1) 4.164(7.8) 3.463, 3.950</td>
</tr>
<tr>
<td>H-4</td>
<td>3.321 3.385 5.377</td>
<td>3.099 3.428 3.405 5.376</td>
</tr>
<tr>
<td>H-5</td>
<td>3.116 3.258 5.554</td>
<td>3.169 3.169 3.268 5.556</td>
</tr>
<tr>
<td>H-6a</td>
<td>3.443 3.530</td>
<td>3.455 3.462 3.538</td>
</tr>
<tr>
<td>H-8 (CH(_3))</td>
<td></td>
<td>1.836</td>
</tr>
</tbody>
</table>

\(^a\) Data were obtained in DMSO-\(d_6\)/2% D\(_2\)O at 55°C. Chemical shifts are referenced to internal tetramethylsilane (set to 0.000 ppm).
Fig. 1

Monosaccharide concentration (mM) vs. Activity (nmol/h)
GlcNAc\(\beta_3\)Man\(\beta_7\)MU (P) + Man\(\beta_7\)MU (S)

\[ \begin{align*}
\beta\text{-Man H-1} & \quad \beta\text{-GlcNAc H-1} \\
S & \quad P \\
\beta\text{-Man H-2} & \quad \beta\text{-GlcNAc H-3 (P)} \\
S & \quad P \\
\beta\text{-GlcNAc H-4,5} & 
\end{align*} \]

MU C-7/\(\beta\text{-Man H-1} \) (C-7 folded)

\[ \begin{align*}
\beta\text{-Man C-3 (P)/}\beta\text{-GlcNAc H-1} & \\
\beta\text{-GlcNAc C-1/}\beta\text{-Man H-3(P)} & 
\end{align*} \]
Fig. 7

A

GlcNAcβ1-3Manβ1-4Glcβ1-1Cer

III II I R

III-1 II-1 II-2 III-6b II-3 III-5+ III-4 R-5 R-4 cis

5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 ppm

B

Manβ1-4Glcβ1-1Cer

II I R

II-1 II-6b II-2 II-3 R-1a I-6a I-6b II-4 II-5 I-2 R-5 R-4 cis

5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 ppm
The drosophila gene brainiac encodes a glycosyltransferase putatively involved in glycosphingolipid synthesis
Tilo Schwientek, Birgit Keck, Steven B. Levery, Mads A. Jensen, Johannes W. Pedersen, Hans H. Wandall, Mark Stroud, Stephen M. Cohen, Margarida Amado and Henrik Clausen
J. Biol. Chem. published online July 18, 2002

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