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Glycomic studies of *Drosophila melanogaster* embryos

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Abstract With the complete genome sequence of *Drosophila melanogaster* defined a systematic approach towards understanding the function of glycosylation has become possible. Structural assignment of the entire *Drosophila* glycome during specific developmental stages could provide information that would shed further light on the specific roles of different glycans during development and pinpoint the activity of certain glycosyltransferases and other glycan biosynthetic genes that otherwise might be missed through genetic analyses. In this paper the major glycoprotein N- and O-glycans of *Drosophila* embryos are described as part of our initial undertaking to characterize the glycome of *Drosophila melanogaster*. The N-glycans are dominated by high mannose and paucimannose structures. Minor amounts of mono-, bi- and tri-antennary complex glycans were observed with GlcNAc and Galβ1–4GlcNAc non-reducing end termini. O-glycans were restricted to the mucin-type core 1 Galβ1-3GalNAc sequence.

Keywords *Drosophila melanogaster* · Mass spectrometry · Glycomics · Sialic acid

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Abbreviations

PNGase F	peptide *N*-glycosidase F
PNGase A	peptide *N*-glycosidase A
MALDI-	Matrix assisted laser desorption ionisation
CAD	collision activated decomposition
GC	gas chromatography
Hex	hexose
HexNAc	*N*-acetylhexosamine
Fuc	fucose
Gal	galactose
GlcNAc	*N*-acetylglucosamine
Man	mannose
NeuAc	*N*-acetylneuraminic acid
NeuGc	*N*-glycolylneuraminic acid
MS	mass spectrometry
MS/MS	tandem mass spectrometry
CNS	central nervous system
GFP	green fluorescent protein

Introduction

A huge quantity of data now exists that irrefutably shows that complex carbohydrates as constituent cell surface glycoconjugates are involved in the mediation of cell-cell interactions, cell adhesion and cell migration. In order to elucidate the role of carbohydrates in such processes their molecular structure requires assignment. This in recent years has led to the development of the new analytical field of glycomics which aims to define the entire set of glycans present in an organism. It is likely that the first organisms for which the glycomes will be defined will be the model organisms such as *Drosophila melanogaster* that are already commonly used by biologists. Comparison of the glycomes of different model organisms and their developmental changes will offer insight into a

possible common (conserved) function for certain groups of glycans [1].

Despite the availability of the complete *Drosophila* genome and a broad range of efficient technical tools on the molecular, genetic and cellular level, relatively little is known about the structure of protein linked glycans. To date the most comprehensive characterization of *Drosophila* N-linked glycans was performed on extracts of whole adult flies [2]. MALDI-TOF MS and HPLC analysis of PNGase A released glycans revealed high levels of high mannose and paucimannose structures. The later could be core fucosylated on either the 3- or 6- position of the core with 1% of the total glycans difucosylated. The most abundant structure (36% of the total) was a $FucMan_3GlcNAc_2$ structure. Trace amounts of mono and bi-antennary N-glycans with non-extended GlcNAc antennae and sub-stoichiometric core fucosylation were also detected.

Drosophila mucin type O-linked glycans appear to be also dominated by simple sructures. Lectin and specific anti-carbohydrate antibody binding experiments on a wide range of *Drosophila* tissues and cells indicates restriction to a mucin-type core 1 (Galβ1-3GalNAc-O-Ser/Thr) structure [3–6].

This apparent lack of complex structures among the N-and O-glycans characterized to date is somewhat surprising in the light of recent findings on the presence of functional glycosyltransferases supposedly involved in the biosynthesis of complex glycoconjugates in *Drosophila*. For example, it was recently demonstrated that *Drosophila* possesses two functional β1,4-N-acetylgalactosaminyltransferases [7] and a vertebrate-type α2-6-sialyltransferase, DSIAT [8], the enzymes potentially participating in the synthesis of complex *N*-linked glycans. A close homologue of one of the *Drosophila* β1,4-N-acetylgalactosaminyltransferases, β4GalNAcT, was also characterized in the lepidopteran insect *T. ni* [9]. The lepidopteran enzyme can modify N-linked glycans, producing GalNAcβ1-4GlcNAc (LacdiNAc)—terminating antennae [9]. Accordingly, Drosophila β4GalNAcT was shown to synthesize LacdiNAc structure *in vitro* [7], while our analyses have demonstrated that LacdiNAc termini of N-linked oligosaccharides are the preferred *in vitro* acceptors of DSAIT [8]. All these data strongly suggest the capability of *Drosophila* cells to produce N-linked glycans including sialylated LacdiNAc structures. Curiously, the *in vivo* presence of sialic acid in *Drosophila* has only been reported in one study [10], which has not been further corroborated. As of now, the identity of sialylated structures in *Drosophila* remains ellusive.

In recent years we have been involved in the development of highly sensitive and rapid mass spectrometric screening strategies for defining the glycosylation repertoires of organs in knockout mice [11,12], *Caenorhabditis elegans* [1,13,14] and mammalian cells [15]. This methodology has also been adopted by The Consortium for Functional Glycomics for its glycan profiling of human and mouse tissues and cells of the immune system [www.functionalglycomics.org]. Here we present the application of this method to the initial characterization of major N-and O-linked glycans produced in *Drosophila* embryos, paying specific attention to potential presence of sialylated structures.

Materials and methods

Drosophila stocks and transgenes

As "wild type" strain, we used Canton S flies carrying w^{1118} marker [16]. C155-GAL4 is a first chromosome GAL4 enhancer-trap insertion in the *elav* locus; it drives the expression of GAL4 activator in all neurons throughout development starting at embryonic stage 12 [17]. The *pUAST-DSiaT-HA* construct was produced by cloning the full-length DSiaT cDNA [8] into *pUAST* vector for *in vivo* ectopic expression [18]. The *DSiaT* cDNA sequence in this construct was fused to a haemagglutinin (HA) tag-coding DNA fragment to produce a C-terminally HA-tagged DSIAT protein [8].

In situ hybridization and GFP microscopy

In situ hybridization of *Drosophila* embryos was performed using as described in [19] with digoxigenin-labeled RNA probe (produced with DIG RNA labeling mix and *DSiaT* cDNA as a template) from (Roche) and anti-DIG antibody conjugated with alkaline phosphatase (Roche). The *in situ* hybridization included negative control staining with a probe transcribed from the corresponding anti-parallel cDNA sequence (data not shown). The expression of mCD8::GFP reporter [20] was analysed in fixed embryos using optical sectioning with Axioplan 2 microscope (Zeiss) equipped with ApoTome module. The 3D reconstruction of GFP expression was obtained using Zeiss AxioVision software.

Drosophila embryo collection

Embryos were collected for 18–23 h from large population cages at $\sim$23°C on apple-juice agar plates supplemented with fresh yeast paste. The collected embryos were transferred onto small sieves, washed extensively with distilled water to remove residual yeast and then stored at -80°C until sample processing.

Detergent extraction of *Drosophila* material

Approximately 500 mg of *Drosophila* embryos were homogenised on ice in an extraction buffer of 0.5% w/v

cetyltrimethylammonium bromide (CTAB), in 0.1 M Tris (pH 7.4), and extracted for a further 24 h at 4°C. Solid debris were removed by centrifugation at 3000 rpm for 10 min. Detergent was removed by extensive dialysis against 50 mM ammonium bicarbonate buffer (pH 7.6).

Reduction and carboxymethylation

Drosophila embryo extracts were reduced in 1 ml of 50 mM Tris-HCl buffer, pH 8.5, containing 2 mg/ml dithiothreitol. Reduction was performed under a nitrogen atmosphere at 37°C for 1 h. Carboxymethylation was carried out by the addition of iodoacetic acid (5-fold molar excess over dithiothreitol), and the reaction was allowed to proceed under a nitrogen atmosphere at 37°C for 1 h. Carboxymethylation was terminated by dialysis against 4 × 2.5 l of 50 mM ammonium bicarbonate, pH 8.5, at 4°C for 48 h. After dialysis, the sample was lyophilized.

Tryptic digest

The reduced carboxymethylated extracted proteins were digested with (TPCK) treated bovine pancreas trypsin (EC 3.4.21.4, Sigma), for 5 h at 37°C in 50 mM ammonium bicarbonate buffer (pH 8.4). The products were purified by C18-Sep-Pak (Waters Corp.) as described [21].

PNGase F digestion

PNGase F (EC 3.2.2.18, Roche Molecular Biochemicals) digestion was carried out in ammonium bicarbonate buffer (50 mM, pH 8.4) for 16 h at 37°C using 0.6 U of the enzyme. The digested sample is loaded on a pre-conditioned C_{18}-Sep-Pak (Waters Corp.) and eluted with 5 ml of 5% acetic (the *N*-glycan fraction) followed by elution with 4 ml of 20% propanol in 5% acetic acid (the peptide/O-linked glycopeptide fraction).

PNGase A digestion

Glycopeptides remaining after PNGase F digestion were further digested with PNGase A (EC 3.5.1.52, Roche Molecular Biochemicals), in ammonium acetate buffer (50 mM, pH 5.0), for 16 h at 37°C using 0.2 mU of the enzyme. The released N-glycans were purified on a C_{18}-Sep-Pak (Waters Corp.) as described above.

α-mannosidase digestion

The PNGase F released glycans were incubated with α-mannosidase (jack bean, EC 3.2.1.24; Glyko), 0.5 units in 100 μl of 50 mM ammonium acetate buffer, pH 4.6 at 37°C for 48 h with a fresh aliquot of enzyme added after 24 h.

Reductive elimination

O-glycans were released by reductive elimination in 400 μl of sodium borohydride (38 mg/ml in 0.05 M sodium hydroxide) at 45°C for 16 h. Reactions were terminated by dropwise addition of glacial acetic acid, followed by Dowex 50W-X8 (H) 50–100 mesh (BDH, Poole, U.K.) chromatography and borate removal.

Chemical derivatisation for MALDI-MS and GC-MS

Permethylation using the sodium hydroxide procedure was performed as described [21]. After derivatisation the reaction products were purified on C_{18}-Sep-Pak (Waters Corp.) as described [21]. MALDI data were acquired using a Perseptive Biosystems Voyager-DETM STR mass spectrometer in the reflectron mode with delayed extraction. Derivatized glycans were dissolved in 10 μl of methanol, and 1 μl of dissolved sample was premixed with 1 μl of matrix (2,5-dihydrobenzoic acid) before loading onto a target plate.

CAD-ES-MS/MS analysis

CAD-ES-MS/MS spectra were acquired using a Q-TOF (Micromass, Manchester, United Kingdom) instruments. The permethylated glycans were dissolved in methanol before loading into a spray capillary coated with a thin layer of gold/palladium, inner diameter 2 μl (Proxeon, Odense, Denmark). A potential of 1.5 kV was applied to a nanoflow tip to produce a flow rate of 10–30 nl/min. The drying gas used was N_2 and the collision gas was argon, with the collision gas pressure maintained at 10^{-4} millibar. Collision energies varied depending on the size of the carbohydrate, typically between 30 and 90 eV.

GC-MS linkage analysis

Partially methylated alditol acetates were prepared from permethylated samples for gas chromatography-MS linkage analysis. Briefly, the permethylated glycans were hydrolyzed with 2 M trifluoroacetic acid for 2 h at 121°C, reduced with 10 mg/ml sodium borodeuteride in 2 M aqueous ammonium hydroxide at room temperature for 2 h, and then acetylated with acetic anhydride at 100°C for 1 h. Linkage analysis was carried out on a Perkin Elmer Clarus 500 instrument fitted with an RTX-5 fused silica capillary column (30 m × 0.32-mm internal diameter; Restek Corp.). The sample was dissolved in hexanes and injected onto the column at 65°C. The column was maintained at this temperature for 1 min and then heated to 290°C at a rate of 8°C/min.

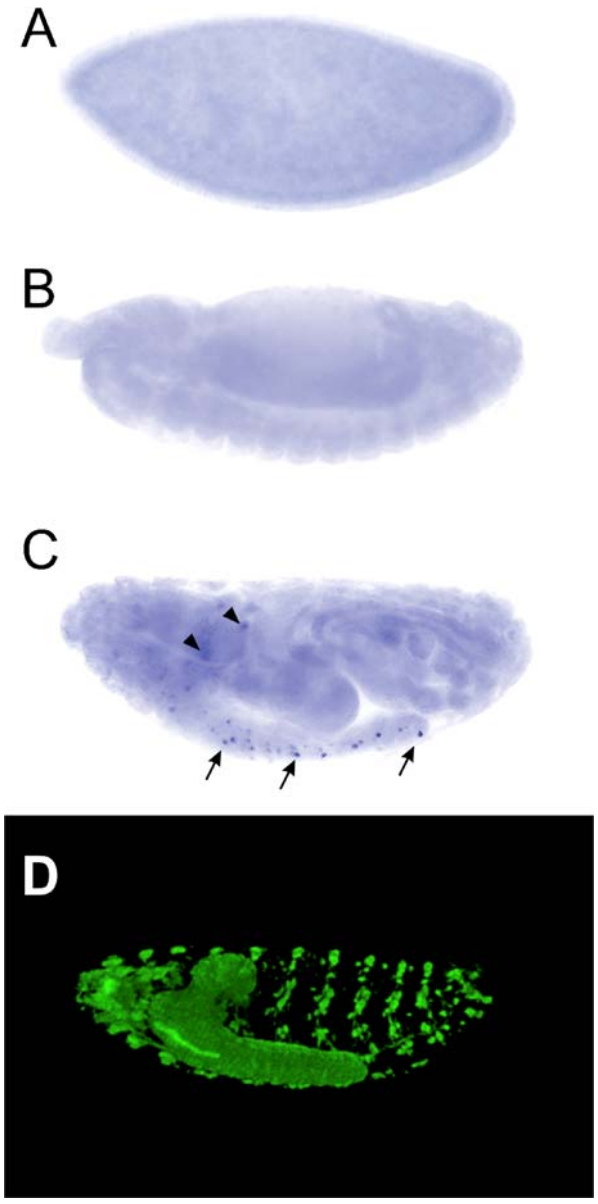

Fig. 1 Endogenous and ectopic expression of *DSiaT* in Drosophila embryos. **A**–**B**, *DSiaT* expression as revealed by *in situ* hybridization. No *DSiaT* transcript is detected in stage 5 (A) or 13 (B) embryos. **C**: during late embryogenesis (stage 17) *DSiaT* is expressed in a number of cells within the CNS, including brain (arrowheads) and ventral ganglion (arrows) regions. **D**: The pattern of ectopic expression in C155-GAL4 embryos (stage 17) visualized using UAS-mCD8::GFP reporter construct. GFP fluorescence highlights the peripheral and central system neurons. The image represents a 3D-reconstruction of GFP expression from a series of optical sections. In all images, anterior is to the left. B-D, lateral view.

Results

Producing DSIAT-overexpressing transgenic flies

In situ hybridization analysis of embryonic expression revealed very restricted pattern of DSiaT expression during embryogenesis (Fig. 1A–C and [8]). No DSiaT expression was detected from early embryonic stage 5 until stage 15 (Fig. 1A and B). During late embryogenesis (stages 16–17), DSiaT mRNA was detected in a limited number of cells within the developing CNS, both in the ventral ganglion and brain hemispheres (Fig. 1C). Although the number of DSiaT-expressing cells (presumably neurons) gradually increases in the CNS during embryonic and following larval stages (data not shown), these cells account for only a small fraction of total *Drosophila* CNS. Thus, in order to facilitate our downstream analysis of sialylated structures, we decided to overexpress DSiaT in *Drosophila* embryos using a pan-neuronal C155-GAL4 driver [17,18]. Our rational was also based on the assumption that active donor sugar (CMP-Sia) and acceptor (LacdiNAc)-producing biosynthetic pathways are present in *Drosophila* neurons. This assumption is indirectly supported by the fact that genes encoding functional β1-4GalNAc-transferases and Neu5Ac phosphate synthase (one of the key enzymes in the biosynthesis of sialic acid) are ubiquitously expressed throughout embryogenesis [7,23]. For overexpression experiments, the transgenic insertion of pUAST-DSiaT-HA construct on the 3rd chromosome (designated as 101.2) was produced by P-element mediated transformation [33] and combined with the C155-GAL4 driver (on the X chromosome) which induces expression of UAS-controlled transgenes in all *Drosophila* neurons (Fig. 1D). The flies of the resulting transgenic strain, C155-GAL4; 101.2, were morphologically indistinguishable from wild type flies. We used this strain for the large-scale collection of DSiaT-overexpressing embryos for subsequent glycan analysis.

MALDI-MS of PNGase F released N-glycans from *Drosophila* wild type embryos

As we have previously demonstrated as part of our characterisation of N-glycosylation in nematode species the use of sequential digestion of extracted glycopeptides with PNGase F and PNGase A allows a partial fractionation of released N-glycans on the basis of whether they have a fucose attached to the 3-position of the Asn-liked GlcNAc residue [24]. Released glycans were permethylated to increase the sensitivity of detection and to direct the subsequent MS/MS fragmentation. The spectra of the PNGase F released glycans (Fig. 2A and Table 1) of wild type *Drosophila* embryos are dominated by signals consistent with high mannose type glycans *m/z* 1580–2397 $Man_5GlcNAc_2$- $Man_9GlcNAc_2$. There are also additional signals at *m/z* 2601–2805 $Hex_{10}GlcNAc_2$-$Hex_{11}GlcNAc_2$ indicating Hex addition. Paucimannose N-glycans *m/z* 967–1550 $Man_2GlcNAc_2$-$FucMan_4GlcNAc_2$ with core fucosylation are also seen. Minor amounts

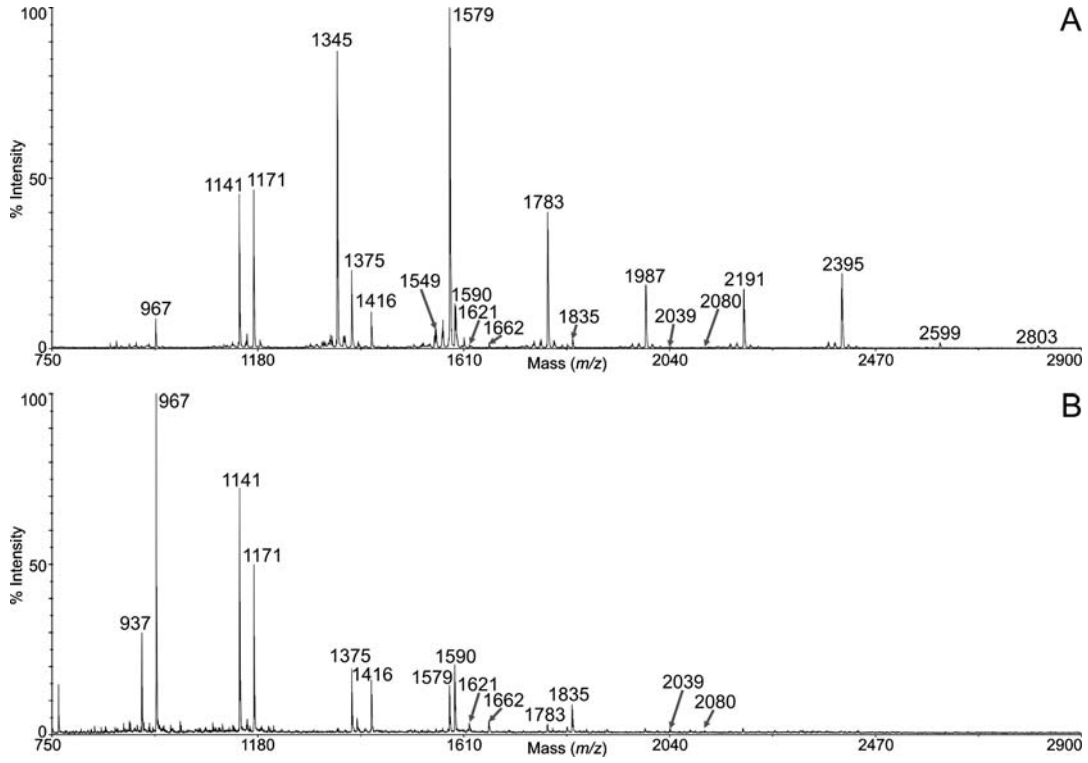

Fig. 2 MALDI spectra of permethylated N-glycans released with PNGase F from *Drosophila* wild type embryos (A) before and (B) after digestion with α-mannosidase.

Table 1 Assignments of molecular ions observed in MALDI spectra of permethylated N-glycans of *Drosophila* embryos

Signal (m/z)	Assignment
967	$Hex_2HexNAc_2+Na^+$
1141	$FucHex_2HexNAc_2+Na^+$
1171	$Hex_3HexNAc_2+Na^+$
1315	$Fuc_2Hex_2HexNAc_2+Na^{+*}$
1345	$FucHex_3HexNAc_2+Na^+$
1375	$Hex_4HexNAc_2+Na^+$
1416	$Hex_3HexNAc_3+Na^+$
1519	$Fuc_2Hex_3HexNAc_2+Na^{+*}$
1549	$FucHex_4HexNAc_2+Na^+$
1579	$Hex_5HexNAc_2+Na^+$
1590	$FucHex_3HexNAc_3+Na^+$
1621	$Hex_4HexNAc_3+Na^+$
1662	$Hex_3HexNAc_4+Na^+$
1783	$Hex_6HexNAc_2+Na^+$
1835	$FucHex_3HexNAc_4+Na^+$
1987	$Hex_7HexNAc_2+Na^+$
2039	$FucHex_4HexNAc_4+Na^+$
2080	$FucHex_3HexNAc_5+Na^+$
2191	$Hex_8HexNAc_2+Na^+$
2395	$Hex_9HexNAc_2+Na^+$
2599	$Hex_{10}HexNAc_2+Na^+$
2803	$Hex_{11}HexNAc_2+Na^+$

[*] PNGase A only.

of complex type N-glycans are seen at *m/z* 1417–2081 $Hex_3HexNAc_3$-$FucHex_3HexNAc_5$.

Linkage analysis of *Drosophila* embryo PNGase F released N-glycans

Drosophila PNGase F released N-glycans from wild type embryos were subjected to linkage analysis (Table 2). These results are fully consistent with high mannose and paucimannose structures being the major constituents of the N-glycan population, as terminal mannose is the most abundant residue. The low abundance of complex structures is indicated by the presence of small amounts of terminal GlcNAc and terminal Gal. The presence of 2,6-linked Man indicates that some complex glycans are di-antennery on the 6-linked mannose of the trimannosyl core. The presence of terminal Fuc and 4,6-GlcNAc is consistent with core-linked fucose. No signals consistent with terminal GalNAc and therefore LacdiNAc structures were observed.

MALDI-MS of α-mannosidase digested *Drosophila* wild type embryo PNGase F released N-glycans

In all N-glycan structures characterised to date sialic acid residues are only found on complex and hybrid type glycans.

Table 2 GC-MS analysis of partially methylated alditol acetates obtained from the PNGase F released N-glycans of wild type *Drosophila* embryos

Elution time (mins)	Characteristic fragment ions	Assignment	Relative abundance
17.44	115, 118, 131, 162, 175	terminal fucose	0.082
18.96	102, 118, 129, 145, 161, 205	terminal mannose/glucose	1.000
19.21	102, 118, 129, 145, 161, 205	terminal galactose	0.010
20.11	129, 130, 161, 190	2-linked mannose	0.422
20.30	101, 118, 129, 161, 234	3-linked mannose	0.054
20.53	102, 118, 129, 162, 189, 233	6-linked mannose	0.099
21.69	129, 130, 189, 190	2,6-linked mannose	0.142
21.85	118, 129, 189, 234	3,6-linked mannose	0.595
22.77	117, 159, 203, 205	terminal GlcNAc	0.007
23.67	117, 159, 233	4-linked GlcNAc	0.174
24.98	117, 159, 261	4,6-linked GlcNAc	0.015

As seen in Fig. 2A these classes of glycans are only minor components compared to the dominant high mannose type structures. Therefore to reduce the abundance of high mannose structures (and also to confirm them as high mannose structures) and enhance the detection of potential sialylated complex and hybrid glycans the PNGase F released glycans were digested with α-mannosidase. As can be seen in Fig. 2B a clear reduction in signals of *m/z* 1580–2397 can be observed with a concurrent increase in signals consistent with the core of the digested N-glycans *m/z* 763–967 $ManGlcNAc_2$-$FucMan_2GlcNAc_2$ confirming them as high mannose type N-glycans. Complex type N-glycans are easily observed at m/z 1417–2081 $Hex_3HexNAc_3$-$FucHex_3HexNAc_5$ but again no signals with compositions consistent with sialylated components were observed.

ES-MS-MS of α-mannosidase digested *Drosophila* wild type embryo PNGase F released N-glycans

The sequence of the complex glycans were defined by collisionally activated decomposition (CAD) tandem mass spectrometry (MS/MS) using a nanoelectrospray instrument. Fig. 3 shows the MS-MS spectra obtained from the fragmentation of the $[M + 2Na]^{2+}$ molecular ion of the complex N-glycan of composition $FucHex_3HexNAc_4$. The key fragment ions at *m/z* 474 and 1385 indicate core fucosylation, whilst those at *m/z* 282, 1318 and 1577 are consistent with bi-antennary glycans with single HexNAc antennae. Similar experiments on the complex N-glycan of composition $FucHex_4HexNAc_4$ produced a key fragment ion at *m/z* 486 indicating the presence of a LacNAc (Galβ1-4GlcNAc) type antennae (data not shown). No evidence for LacdiNAc structures was obtained.

MALDI-MS of *Drosophila* wild type embryo PNGase A released N-glycans

The spectra of the PNGase A released glycans (Fig. 4 and Table 1) of wild type, *Drosophila* embryos are again dominated by signals consistent with high mannose type glycans *m/z* 1580–2397 $Man_5GlcNAc_2$-$Man_9GlcNAc_2$. Such glycans are sensitive to PNGase F digestion and their presence in the PNGase A pool of N-glycans indicates their incomplete digestion by PNGase F and again indicates their high abundance. New signals at *m/z* 1316–1520, $Fuc_2Man_{2\text{-}3}GlcNAc_2$ indicate the presence of paucimannose N-glycans with core di-fucosylation.

ES-MS-MS and linkage analysis of *Drosophila* wild type embryo PNGase A released N-glycans

In order to confirm the presence of core di-fucosylated N-glycans the $[M + 2Na]^{2+}$ molecular ion of the N-glycan of composition $Fuc_2Hex_3HexNAc_2$ was subjected to ES-MS-MS analysis. Key fragment ions at *m/z* 648 and 894 definitively identify that the reducing end GlcNAc of the chitobiose core is substituted with two fucose residues. This was also confirmed from GC-MS linkage analysis of the PNGase A released glycans which contained a 3,4,6-linked GlcNAc residue (data not shown).

MS analysis of *Drosophila* wild type embryo O-glycans

O-glycans were chemically released by reductive elimination prior to permethylation and MALDI-MS analysis. The spectra of wild type *Drosophila* embryo O-glycans are dominated by a signal at *m/z* 534 consistent with a composition of HexHexNAc. An additional very minor signal at *m/z* 330 consistent with a composition of HexNAc was also observed. No signals with compositions consistent with sialylated components were observed. The major O-glycan was also subjected to ES-MS/MS to confirm the arrangement of the disaccharide. Major fragment ions at *m/z* 259 and 298 are fully consistent with a non-reducing end terminal Hex residue linked to the 3 position of a HexNAcitol. GC-MS linkage analysis of the O-glycans indicated the presence of terminal Gal and 3-linked GalNAcitol residues (data not shown). Taken together these data are fully consistent with the major O-glycan in

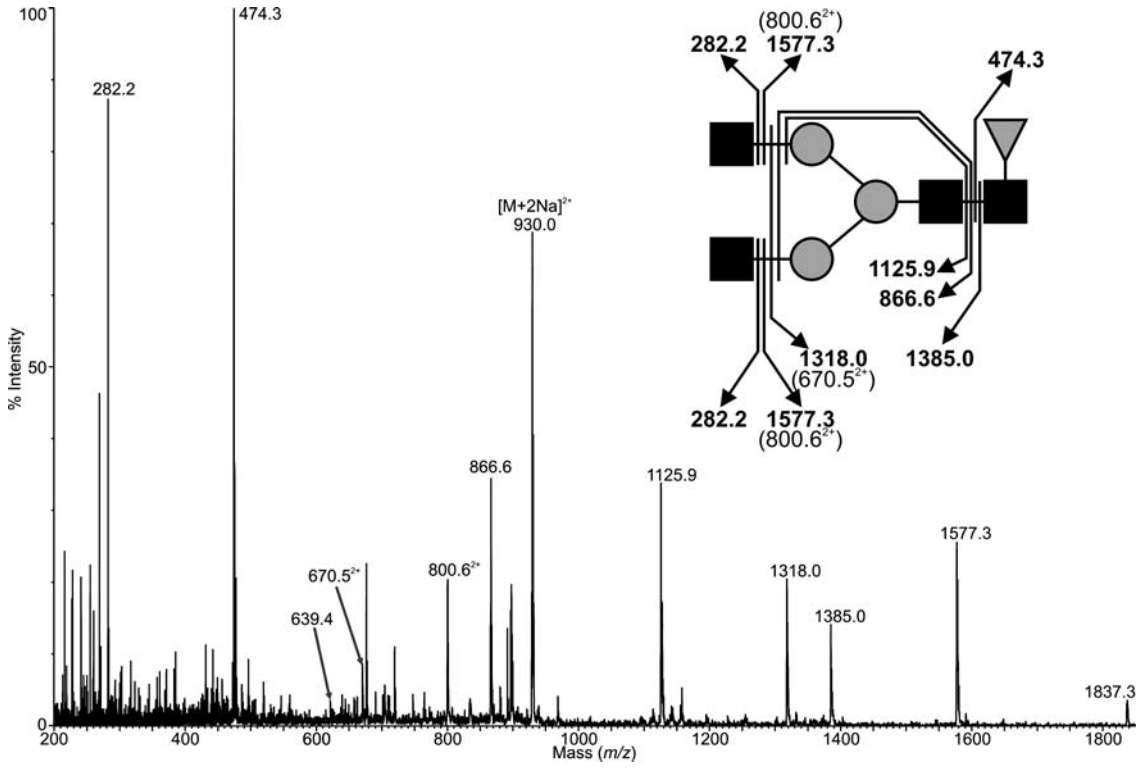

Fig. 3 ES-MS/MS spectra of the $[M + 2Na]^{2+}$ molecular ion of the complex N-glycan of composition $FucHex_3HexNAc_4$ derived from the permethylated N-glycans released with PNGase F from *Drosophila* wild type embryos after digestion with α-mannosidase.

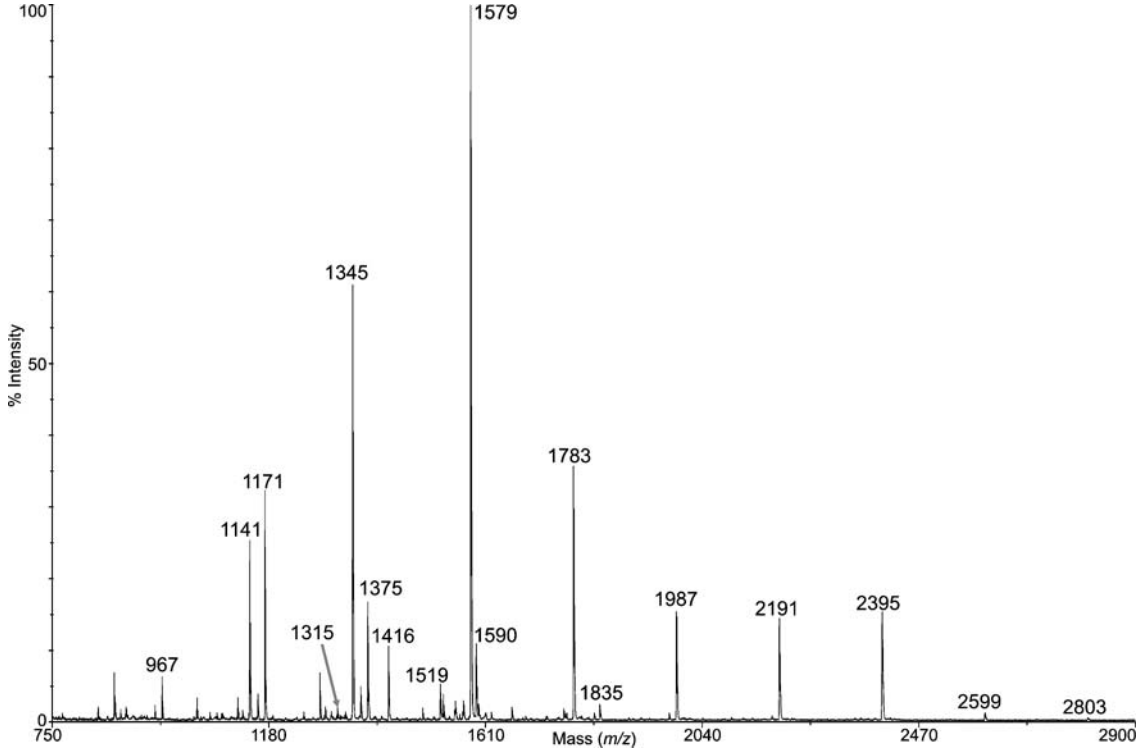

Fig. 4 MALDI spectra of permethylated N-glycans released with PNGase A from *Drosophila* wild type embryos.

Drosophila embryos being a core type 1 Galβ1-3GalNAc structure.

Taken together these data allow the *Drosophila* embryo N- and O-glycan structures to be proposed in Fig. 5.

Analysis of *C155-Gal4; 101.2 Drosophila* embryos

N- and O-linked glycans were released from *C155-Gal4; 101.2 Drosophila* embryos and analysed by a similar mass spectrometric strategy to that described for *Drosophila* wild type (w^{1118}) embryos above. In all experiments the data produced was remarkably similar to that of the wild type with no evidence of sialylated glycans being observed (data not shown).

Discussion

The data presented represent the most comprehensive glycomic characterization of protein glycosylation in *Drosophila* embryos to date. Our results indicate that N-glycosylation is dominated by oligomannosidic and paucimannosidic structures that are often core-fucosylated at both the 3- and 6-position and can be di-fucosylated. The detection of di-fucosylated core structures confirm the observations of Fabini *et al.* [2], who also detected similar structures in whole adult *Drosophila* N-glycans. The α1-3 linked core fucose was also demonstrated to be responsible for the binding of anti-horseradish peroxidase antibodies to *Drosophila* embryonic nervous system tissue. We also detected mono-, bi- and tri-antennary complex glycans in very small amounts. The antennae are usually not extended beyond the first GlcNAc but we have observed evidence via MALDI-MS composition, GC-MS linkage analysis and ES-MS-MS of a LacNAc (Galβ1-4GlcNAc) type antenna. The presence of 2,6- linked Man indicates that some complex glycans are di-antennery on the 6-linked mannose of the trimannosyl core. This is the first description of such a structure in *Drosophila*. The data also indicates that homologs of GlcNAcT I, GlcNAcT II, GlcNAcT V, must be active in *Drosophila* embryos. Previos BLAST searching of the *Drosophila* genome has revealed the presence of potential homologs of GlcNAc T I, II, III, IV and VI but not GlcNAc T V although 3 homologs of other β1-6 GlcNAc transferases were present [25] and an active GlcNAc T V enzyme has been described in *C. elegans* [26]. The lack (or very low abundance) of complex structures is reminiscent of the *C. elegans* glycome [1,13,14], indicating that the recently described *Drosophila* glycosyltransferases (such as sialyltransferase and *N*-acetylgalactosaminyltransferase) [7,8] modify only a small proportion of the N-linked glycans. This is most likely due to the presence in both insects and *C. elegans* of a membrane bound β-*N*-acetylglucosaminidase which can act on remove the β1-2 GlcNAc residue added by GlcNAcT1 and thus prevent complex glycan formation [27,28].

We did not detect sialylated structures in the entire embryonic N-glycome, even in DSiaT overexpressing embryos. This is consistent with additional experimental approaches, including GC-MS monosaccharide composition analysis, which also failed to detect any trace of sialic acids (data not shown). This result could be explained by the temporally and/or spatially restricted activity of the corresponding donor sugar-producing pathway, as well as by the low abundance of the acceptor, possibly represented by N-linked LacdiNAc termini. Thus, low abundant sialylated structures might be challenging to detect in the pool of dominating oligomannosidic and paucimannosidic glycans. Enrichment

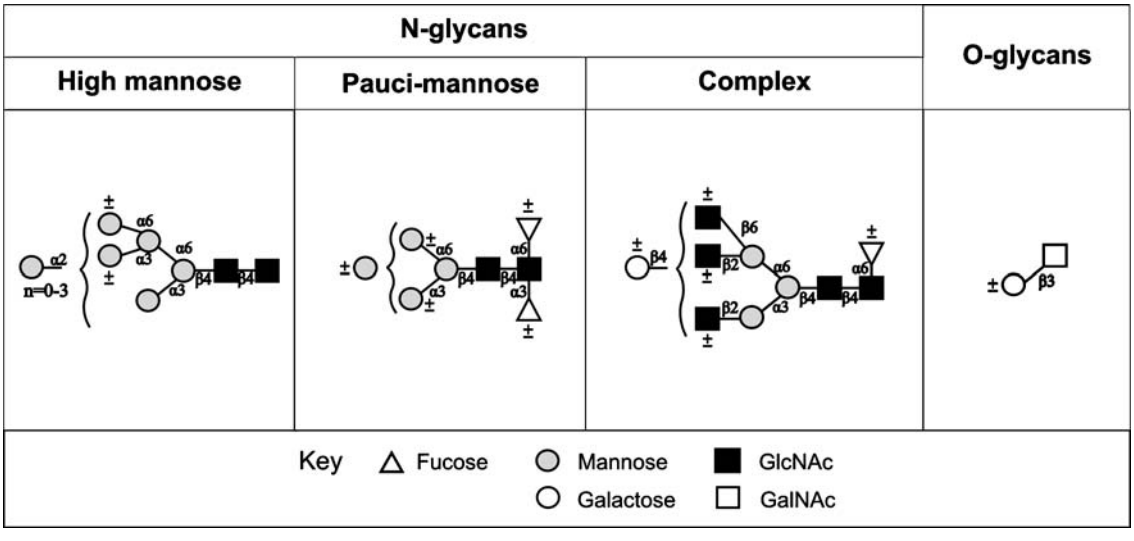

Fig. 5 Proposed structures of the major N- and O-linked glycans of *Drosophila* embryos.

and/or fractionation steps should be implemented to enrich for these low-abundance glycans with non-mannose terminating structures. Two approaches, currently tested in our laboratory, include tissue sub-fractionation and lectin-affinity chromatographic fractionation on a set of complementary lectin columns. Yet another possibility of increasing the sensitivity of sialic acid detection would be the metabolic labeling of sialylated structures by using chemically modified sialic acid precursors (such as ManNAz, [29]), however this technique has not yet been adapted for *Drosophila*.

Another aspect of the *Drosophila* glycomics project is the characterization of O-linked glycome. We have shown that the major mucin type O-glycan found in the *Drosophila* embryos is a core type 1 Galβ1-3GalNAc structure. This is consistent with analysis of the *Drosophila* genome which indicate that up to 14 members of the UDP-GalNAc:Polypeptide *N*-Acetylgalactosaminyltransferase (ppGaNTase) family may be present which, like their mammalian homologs, vary in their substrate specificity and tissue distribution [30]. Recently three active *Drosophila* core 1 β1-3 galactosyltransferases have also been characterised [31].

O-linked fucose represents another important modification that is found on several *Drosophila* glycoproteins [32]. O-linked fucose plays important developmental roles in a wide variety of species, from *Drosophila* to mammals, with *Drosophila* representing an important model system for studying the molecular mechanisms of corresponding developmental processes [32,33]. At the same time, the structure of O-linked fucose glycan has not been fully characterized in *Drosophila* development. Experiments are underway to characterize this class of O-glycans as well.

Thus, surveying the *Drosophila* N- and O-glycome would provide much needed data and complement the available proteome and genome information, facilitating our better understanding of the molecular bases of important developmental processes.

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