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Monosaccharide compositions of *Danaus plexippus* (monarch butterfly) and *Trichoplusia ni* (cabbage looper) egg glycoproteins

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Monosaccharide compositions of eggs from *Danaus plexippus* (monarch butterfly) and *Trichoplusia ni* (cabbage looper) were analyzed. Analyses were performed mainly with high performance anion exchange chromatography (HPAEC) using crude extracts of eggs or SDS-PAGE separated and PVDF-blotted protein bands. Man and GlcN were the major components in all cases, but low levels of Gal and Fuc were possibly present in some samples. Some *T. ni* egg glycoproteins even contained GalN. Although a peak comigrating with Neu5Ac could be detected with HPAEC-PAD or RP-HPLC (fluorometry) after derivatization with 1,2-diamino-4,5-methylenedioxy-benzene, the quantities were too small to be significant as an integral part of the analyzed glycoproteins. These data suggests that most of glycans on the glycoproteins are pauci-Man type N-glycans, but a small portion of N-glycan may be either hybrid type or complex type.

Keywords: **insect eggs, monarch butterfly, cabbage looper, glycoproteins**

Introduction

Earlier studies showed that most vertebrate recombinant glycoproteins, when produced by baculovirus expression vectors in insect cell culture, possess only oligo-mannose type or paucimannoside oligosaccharides in contrast to the complex-type glycans often dominant in vertebrate glycoproteins. However, recently, several studies with either native insect proteins or recombinant proteins expressed in insects suggested that insect cells have capacity to synthesize hybrid and complex oligosaccharides (for reviews, see März et al., 1995). For example, some of the N-glycans from honeybee venom phospholipase A_2 (PLA_2) were found to contain a fucosylated LacdiNAc [GalNAcβ1-4(Fucα1-3)GlcNAc] moiety and α-1,3- and α-1,6-linked fucoses at the same innermost GlcNAc, giving rise to difucosylated structures (Hard et al., 1993; Kubelka et al., 1993). Studies with native proteins from three insect cell lines, *Mamestra brassicae* (MB-0503), *Spodoptera frugiperda* (Sf21), and *Bombyx mori* (Bm-N), showed significant differences in the degree of α1,3-fucosylation and occurrence of a small percentage of hybrid N-glycans (Kubelka et al.,1994; Staudacher et al., 1992). The presence of sialic acids has been reported only for recombinant alkaline phosphatase produced from *T. ni* larvae (Davis and Wood, 1995) and in recombinant plasminogen produced in insect cell cultures (Davidson and Castellino, 1991a; Davidson and Castellino, 1991b; Davidson et al., 1990).

More recently, variability in glycosylation in different insect cells and different developmental stages within an insect has become increasingly evident. Analysis of N-glycans of recombinant interferon-γ secreted from *Estigmena acrea* (Ea-4) and *S. frugiperda* (Sf-9) cell lines showed that while the Ea-4 cells produced multiple complex and hybrid glycoforms, the Sf-9 cells produced only hybrid N-glycans (Ogonah et al., 1996). It was also reported that lepidopteran larvae glycosylates recombinant proteins produced by baculovirus expression system differently from their tissue culture cells (Lucnow, 1994). More interestingly, the secreted and intracellular murine IgGs produced from *T. ni* (TN-5B1-4) cells were found to contain significantly different types of N-glycans. The intracellular IgG contained only the oligo-mannose type structures, whereas the secreted IgG contained complex, hybrid, oligo-mannose, and paucimannose-type oligosaccharides (Hsu et al., 1996).

To gain further insight into the variability in intrinsic glycosylation in insect, we examined the sugar compositions of glycoproteins in the eggs of lepidopteran insects, *Trichoplusia ni* (cabbage looper) and *Danaus plexippus* (monarch butterfly).

Materials and methods

Materials

Insect eggs were obtained from laboratory-reared colonies of *Trichoplusia ni* (Hughes et al., 1986) and *Danaus plexippus* (Hughes et al., 1993). To attain the uncontaminated outer surface, the eggs were washed for 4 min in 2% chlorox to remove the chorion. They were then washed for 10 min in deionized water and stored at −20°C. The following items were purchased from the sources indicated: reverse-phase C18 Sep-Pak cartridge from Waters (Millipore, Milford, MA); Bakerbond C18 Spe cartridges from J. T. Baker Chem. Co. (Phillipsburg, NJ); Centricon tubes from Amicon (Beverly, MA); 1,2-diamino-4,5-methylenedioxy-benzene (DMB) from Fluka Chemie (Steinheim, Switzerland); trifluoroacetic acid (TFA) from Aldrich (Milwaukee, WI); sodium dodecyl sulfate, γ-aminobutyric acid (GABA), phenylisothiocyanate (PITC) and 5-*N*-glycolylneuraminic acid (Neu5Gc) from Sigma (St Louis, MO); Immobilon-P PVDF membrane from Millipore Co. (Bedford, MA); microspin filters (0.45 μm) from PGC Scientifics (Frederick, MD); GF/C glass fibre filter from Whatman (Clifton, NJ). 2-Keto-3-deoxy-D-*glycero*-D-*galacto*-nanonoic acid (KDN) was synthesized in our laboratory (Y.C.L.). All other chemicals, reagents, and solvents were obtained from available commercial sources and were of ACS grade or higher purity.

General methods

All the eluent solutions for HPLC were vacuum-filtered through a Super R-200 membrane (47 mm, 0.2 μm, Gelman Science) for aqueous solutions or a FP-Vericel^{TM} membrane (47 mm, 0.2 μm, Gelman Science) for solutions containing organic solvents. The C18 Sep-Pak cartridge and C18 Spe cartridges were conditioned by washing with MeOH and then with water. Unless otherwise stated, all evaporations were either by rotary evaporation or with a SpeedVac (Savant, Holbrook, NY).

Analytical methods

Protein quantification

Micro-BCA assay was carried out according to the manufacturer's instruction (Pierce), using bovine serum albumin as a standard (measured with a Shimadzu UV-160U spectrophotometer).

High performance anion-exchange chromatography and PAD

The HPAEC/PAD system consisted of a Bio-LC (Dionex, Sunnyvale, CA) equipped with a pulsed amperometric detector (PAD-II) using an organic-solvent compatible detector cell with a gold working electrode. The detector sensitivity was set at 1000 nA. An autosampler (Spectra System AS3000, Thermo Separation Products, San Jose, CA) was used for sample injection. Data were collected using Dionex ACI (advanced computer interface) and analyzed with Dionex AI-450 software. All the mobile phase buffers were sparged and pressured under helium using an eluent degas module (Dionex).

Total carbohydrate analysis

Phenol-H_2SO_4 method was performed as described (McKelvy and Lee, 1969), and measured with a Spectronic 20 (Bausch and Lomb) colorimeter.

Monosaccharide analysis using HPAEC/PAD

Monosaccharide compositions were analyzed by HPAEC/PAD after hydrolysis of samples with 2 M TFA at 100°C for 4 h for neutral sugars and with 4 M HCl at 100°C for 6 h for amino sugars (Fan et al., 1994a). Each acid-hydrolyzed sample was dissolved in water and filtered through a 0.45 μm nylon microspin filter before injection onto the column. A mixture of Fuc, 6-*O*-methyl-D-galactose, GalN, GlcN, Gal, Glc, and Man was used as standards. Neutral and amino sugars were eluted isocratically for 20 min from a CarboPac PA-1 column (4 × 250 mm) at 1.0 ml/min with the following eluent: 9% of 200 mM NaOH (E2), 91% of water (E3). The column was reconditioned with 100% of E2 for 5 min, followed by a 15-min equilibration with the starting eluent. The detector settings were as follows: E1 − 0.05 V, t_1 − 420 ms; E2 = 0.65 V, t_2 = 180 ms; E3 = −0.1 V, t_3 = 360 ms, with the output range of 1 μA. For the analyses of crude protein preparations, about 1 mg sample was accurately weighed and hydrolysed.

For sialic acid(s) analysis, the sample was eluted through the column at a flow rate of 1.0 ml/min in an isocratic mode using 45% of E2, 50% of E3, and 5% of 1 M sodium acetate/200 mM NaOH (E4). The column was reconditioned, after 20 min, with 50% of E3 and 50% of E4 for 10 min, followed by a 10-min equilibration with the starting eluent. 5-N-Acetylneuraminic acid (Neu5Ac) was used as a reference. The detection limit for Neu5Ac by HPAEC/PAD was about 5 pmoles.

Sialic acid analysis by HPLC of DMB derivatives

To determine the presence of sialic acids in egg proteins, samples were hydrolyzed with 200 μl of 2 M acetic acid at 80°C for 3 h on a heating block. Acid was removed on a SpeedVac at room temperature and the dried residue was dissolved in 300 μl water, and filtered through a Centricon 10 (MWCO 10,000) by centrifuging for 1.5 h at 12,100 × *g*. The filtrates were passed through a 1 ml column of Dowex 50 × 2 (H^+) and the resin was washed twice with 4 ml of water. The flow-through and the washes were combined and freeze-dried.

The dried material was derivatized with DMB as follows (Hara et al., 1989): The sample was dissolved in 100 μl of 2 M acetic acid, incubated with 100 μl of DMB solution (7 mM of DMB dihydrochloride in 1.4 M acetic acid containing 0.75 M β-mercaptoethanol and 18 mM sodium hydrosulfite) for 2.5 h

at 50°C in the dark, cooled in ice water, and then stored at −20°C in the dark for subsequent analysis. A mixture of sialic acids, Neu5Ac, Neu5Gc, and KDN was similarly derivatized with DMB and used as standards.

HPLC analyses of DMB-derivatized sialic acid(s) were performed with a Gilson 715 system equipped with a Rheodyne 7161 syringe-loading sample injector valve (200-µl loop), equipped with a Shimadzu Shim-Pack CLC-ODS (150 × 6 mm i.d.; particle size, 5 µm) column. The column was eluted with a mixture of acetonitrile–methanol–water (9:7:84, v/v/v) at a flow rate of 1 ml/min. The detection was with a Perkin-Elmer LS 40 fluoromonitor using an attenuation factor of 2048, with $\varepsilon_{ex} = 373$ nm and $\varepsilon_{em} = 448$ nm. Neu5Ac, Neu5Gc, and KDN derivatives clearly separated from each other within 15 min of elution.

SDS-PAGE and protein transfer to PVDF membranes

SDS-PAGE was performed by the discontinuous buffer system (Laemmli, 1970) with 12% running gel and 4% stacking gel containing 0.1% SDS in mini-gels (12 × 10 cm, 0.75 mm thickness). The gels were run in a Bio-Rad Mini Protein^{TM} II unit at constant amperage (25 mA) at room temperature, using broad-range proteins (Bio-Rad) as molecular weight standards. The resolved proteins on the gel were stained with 0.1% Coomassie Brilliant Blue R-250 in 40% MeOH/10% acetic acid. The gels were dried for 1 hr under vacuum with heating on a Bio-Rad gel dryer.

For protein transfer, Immobilon-P membranes were soaked in 100% MeOH for a few seconds, and then equilibrated for 20–30 min in blotting buffer (3-[cyclohexylamino]-1-propane-sulfonic acid buffer, pH 10.5) containing 10% MeOH. Gels were removed from the electrophoresis cell and equilibrated in blotting buffer for 5 min. Protein transfer was performed at a 125-mA constant current for 50 min at room temperature. After transfer, the membrane was removed from the sandwich and rinsed with water for 5–10 sec and then saturated with 100% MeOH for a few sec. The membrane was stained with 0.1% Coomassie Brilliant Blue R-250 in 40% MeOH for about 10 sec and de-stained with 50% MeOH for 1–2 min. The transfer efficiency was determined by staining the SDS gel and the back-up sheet with Coomassie B. Blue R-250, after transfer. Under these conditions, proteins with higher M, were transferred to the membrane with minimum loss. Proteins of lower molecular weight passed through the membrane and were lost into the blotting buffer. The membrane was then air-dried and stored at 4°C for subsequent carbohydrate analysis.

Delipidation of egg proteins

Delipidated eggs were used to extract proteins. Eggs were delipidated essentially as described previously (Weiss et al., 1986). One gram (wet weight) of either *T. ni* or *D. plexippus* eggs were homogenized by mild sonication (Bransonic 12) for 30 min in 3 vol of water at 4°C. Ten volumes of MeOH and then 5 volumes of $CHCl_3$ were added to the homogenate while stirring gently at room temperature. After constant stirring for 1 h, the mixture was vacuum-filtered through a glass fibre filter (Whatman GF/C, 4.25 cm). The retentates were homogenized again in 4 vol of water and stirred for 1 hr after addition of 15 vol of chloroform/methanol (1:2, v/v) and filtered as above. The retentates (delipidated eggs) were stored at −20°C for subsequent use for the isolation of egg proteins.

Delipidated eggs were suspended in 5 vol of 6.7 mM Tris buffer, pH 7.0, containing 4 M guanidine hydrochloride and 0.5% Triton X-100, and sonicated on ice for 30 min. The suspension was passed through a GF/C glass fibre filter and the filtrate was dialyzed (MWCO 1,000 tubings, Spectrum, Houston, TX) against running water at 4°C for two days. To remove the residual Triton X-100, the retentate was made to 80% ethanol and allowed to form precipitate at 4°C for one day, centrifuged at 9,000 × *g* for 30 min at 4°C, and the supernatant was discarded. The process was repeated once again, and after centrifugation, the precipitate was dissolved in 10 ml of water, freeze-dried, and stored at −20°C as the crude protein preparations. Each step of the isolation procedures was monitored by micro-BCA assay and phenol-sulfuric acid assay to quantify proteins and carbohydrates, respectively.

Monosaccharide analysis of proteins blotted to PVDF membrane

One mg (as protein) of each egg protein preparation was dissolved in 100 µl of SDS reducing buffer by incubating for 1 hr at 37°C. The protein solution was then heated at 95°C for 4 min and 5 µl was loaded per lane of gel for SDS-PAGE. After transfer to Immobilon-P membrane (Weitzhandler et al., 1994), protein bands were cut out for carbohydrate analyses. Six protein bands of *D. plexippus* (apparent M_r of 180, 73, 60, 50, 40, and 35 kDa) and *T. ni* egg proteins (180, 76, 66, 50, 40, and 34 kDa) from three identical lanes were excised, cut into small pieces and placed into a 1.5-ml microfuge tube (pre-treated with 4 M HCl at 100°C overnight) for 2 M TFA or 4 M HCl hydrolysis. A piece of the clear region of the membrane was used as blank. As an internal standard, 5 µl (0.51 nmol) of 6-*O*-methyl-D-galactose was added to each tube. The microfuge tubes were gently vortexed to soak the excised membrane strips into the acid completely. After hydrolysis, the hydrolysates were transferred to new microfuge tubes and the membrane strips in the original tubes were washed once with 50 µl of water and combined to the hydrolysates. Acids were removed by repeated cycles of dissolving in water and drying in SpeedVac. The dried materials were dissolved in 100 µl of water and passed through a C18 Sep-Pak cartridge (1 ml) and washed with 2 ml of water. The eluates were dried in a SpeedVac, dissolved in 60 µl of water, and filtered through a 0.45-µm nylon microcentrifuge tube filter before analysis.

Quantification of proteins blotted to PVDF membrane

Quantification of proteins blotted to PVDF was done by amino acid analysis as follows: Protein bands were excised as before

and heated at 110°C for 24 h in a 1.5 ml microfuge tube with 400 µl of 6 M HCl. After hydrolysis, the PVDF strips were removed from the tube, and the hydrolysate was dried with SpeedVac without heating. The dried residue was reacted with 20 µl of the PITC solution and incubated at room temperature for 20 min, and dried *in vacuo*. The residue was dissolved in 200 µl of water and aliquots were used for amino acid analysis by HPLC (Ebert, 1986). GABA was added as internal standard (250–500 pmol GABA/10 µl sample) prior to acid hydrolysis.

Results and discussion

For sugar composition analysis, each sample was acid hydrolyzed under two sets of conditions (2 M TFA at 100°C for 4 h for neutral sugars and 4 M HCl at 100°C for 6 h for amino sugars), since each class of sugars has different optimal conditions (Fan et al., 1994b). When high sensitivity is required, sialic acids were analyzed with the DMB derivatization method. Whenever examined, both HPAEC and DMB methods gave the same results. Likewise, sugar composition in a few samples was also analyzed as PMP derivatives (Honda et al., 1989), and the results are indistinguishable with those of HPAEC.

Analysis of crude protein preparation

Delipidation of eggs (1 g each, wet weight) of both insects reduced the weight to 564 mg (*D. plexippus*) and 307 mg (*T. ni*). Extraction of the delipidated eggs with buffered 4 M guanidium hydrochloride/0.5% Triton X-100 yielded crude preparation of egg proteins: 35.1 mg (6.2% of total mass) from *D. plexippus* and 22.9 mg (7.5% of total mass) from *T. ni* eggs.

Typical chromatograms of monosaccharide analysis by HPAEC/PAD are shown in Figure 1. The results showed that crude protein preparations contained Fuc:GalNAc:GlcNAc: Gal:Man in the molar ratio of <0.2:1.4:2.0:<0.4:5.4 for *D. plexippus* and <0.3:5.0:2.0:<1.5:9.5 in *T. ni* egg proteins (Table 1). The presence of large amounts of Glc is probably due to the presence of polysaccharide in the crude protein preparations. The amount of Glc is generally reduced when protein bands on PDVF membranes were analyzed (see below).

Analysis of sialic acids by RP-HPLC of DMB-derivatives showed that *T. ni* egg crude protein preparation contained a peak coincidental with that of standard Neu5Ac (Fig. 2). A similar peak was also observed in *D. plexippus* egg protein preparations (data not shown). However, the levels of the peak co-migrating with Neu5Ac were 9.3 pmol/mg *D. plexippus*

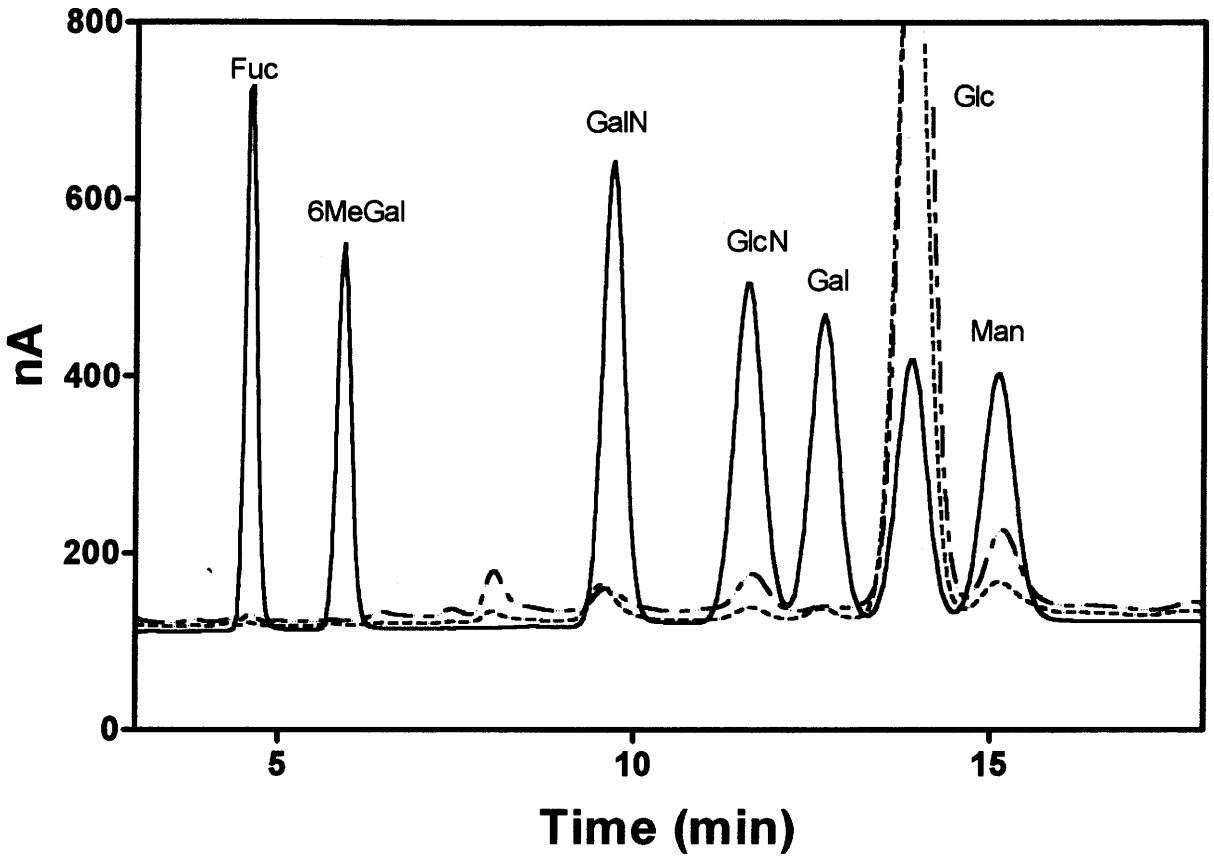

Figure 1. Analysis of neutral and amino sugars in the crude protein preparation of insect eggs by HPAEC/PAD. *T. ni*, short dashes; *D. plexippus*, long dashes; standards, solid line.

Table 1. Sugar composition of crude protein preparations from delipidated insect eggs

	T. ni		*D. plexippus*	
Sugars[a]	*pmol/mg protein*[b]	*molar ratios*	*pmol/mg protein*[b]	*molar ratios*
Fuc	< 38	< 0.3	< 62	< 0.2
GalN	691	5.0	392	1.4
GlcN	276	2.0[d]	560	2.0
Gal	< 203	< 1.5	< 98	< 0.4
Man	1317	9.5	1471	5.4
Neu5Ac[c]	< 17	< 0.08	< 9	< 0.03

[a]HPAEC analysis, except for Neu5Ac.
[b]Determined by BCA.
[c]Determined by DMB-HPLC.
[d]Set GlcN as 2.0.

egg proteins and 17.3 pmol/mg *T. ni* egg proteins (see also Table 1), corresponding to less than 0.05 mol Neu5Ac per mol protein (crude preparation), assuming that actual protein content in the crude protein preparations to be ca. 10% (w/w), and the average glycoproteins in the eggs to be 50 kDa. It is possible that putative Neu5Ac detected in these preparations was from glycolipids or other forms of carbohydrates. There were no detectable peaks corresponding to Neu5Gc and KDN in egg proteins of both species of insects.

SDS-PAGE and transfer of proteins to PVDF membrane

The major protein bands separated on the gel had apparent molecular weights of 180, 73, 60, 50, 40, 35, 27, and 18 kDa

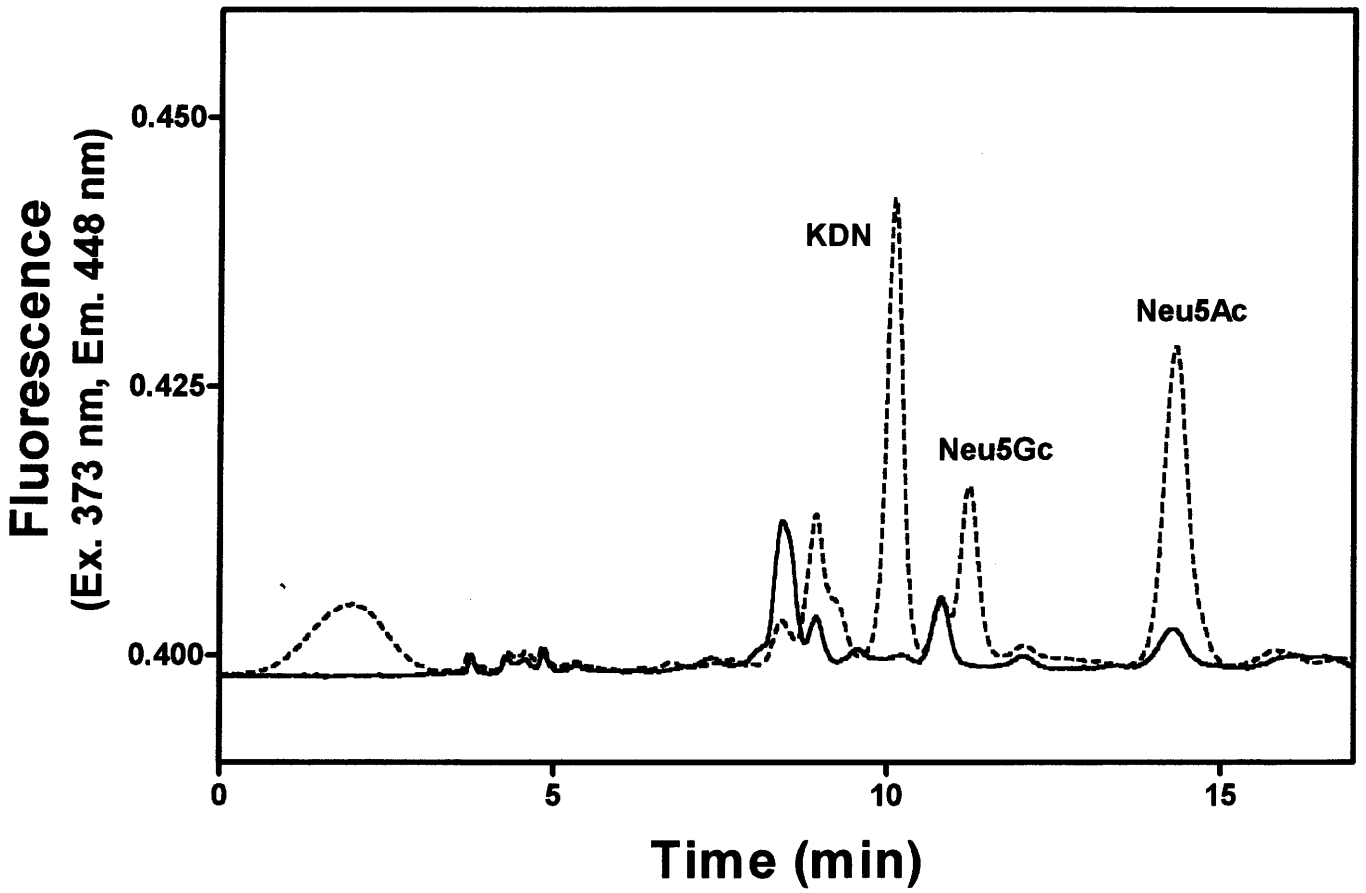

Figure 2. Analysis of sialic acids (as DMB-derivatives) in the crude protein preparation of *T. ni* eggs by RP-HPLC. *T. ni* egg proteins, solid line; sample plus standards, dashed line.

for *D. plexippus* eggs (Fig. 3, lane 1) and 180, 76, 66, 50, 40, 34, 18 kDa (Fig. 3, lane 2) for *T. ni* eggs. Major differences in the protein profile between *D. plexippus* and *T. ni* eggs are the presence of dominant 60 kDa protein in *D. plexippus* eggs and the presence of 66 kDa protein and negligible amount of 27 kDa protein in *T. ni* eggs. Similarly developed gel slab was used without staining for transfer of protein bands to Immobilon-P membrane for carbohydrate analysis of individual protein bands.

Monosaccharide compositions of major egg proteins blotted to PVDF membrane

The blotted major protein bands were analyzed for their monosacchride compositions as described in Methods. As example, chromatograms of neutral sugar analysis of the 60 kDa protein of *D. plexippus* and the 73 kDa protein of *T. ni* eggs are shown in Figure 4. All proteins analyzed had a similar monosaccharide profile in that Man and GlcN were the major constituents, and Gal, GalN, and Fuc were present in much smaller amounts. Results are summarized in Figures 5 & 6.

Of 25 µg of total protein applied per well (determined by BCA assay), the recovery of transferred proteins ranged from 1.2 (50 kDa protein) to 3.4 µg (60 kDa protein) in *D. plexippus* egg proteins and from 0.6 (50 kDa) to 1.8 µg (180 kDa protein) in *T. ni* egg proteins, respectively (determined by amino acid analysis). Calculated carbohydrate contents ranged from 0.9% (40 kDa protein) to 3.2% (180 kDa) in *D. plexippus* egg proteins (Table 2) and 1.3% (34 kDa) to 2.6% (50 kDa) in *T. ni* egg proteins (Table 3). On the assumption that GlcN is present only in N-glycans and each N-glycan contains 2 GlcN residues, most protein bands appear to have at least one N-glycan chain (Tables 2 and 3).

Although the same set of monosaccharides was found in both the crude protein preparations and in the blotted protein bands, there are considerable differences in the sugar composition. In addition to the aforementioned huge Glc peak found in the crude protein preparation, the ratio of Man to GlcN was considerably higher for the crude protein preparations. The Man:GlcN ratios were 3.8:2.0 (180 kDa), 3.7:2.0 (73 kDa), 4.2:2.0 (60 kDa), 3.3:2.0 (50 kDa), 3.2:2.0 (40 kDa), 4.0:2.0 (35 kDa) for blotted protein bands of *D.*

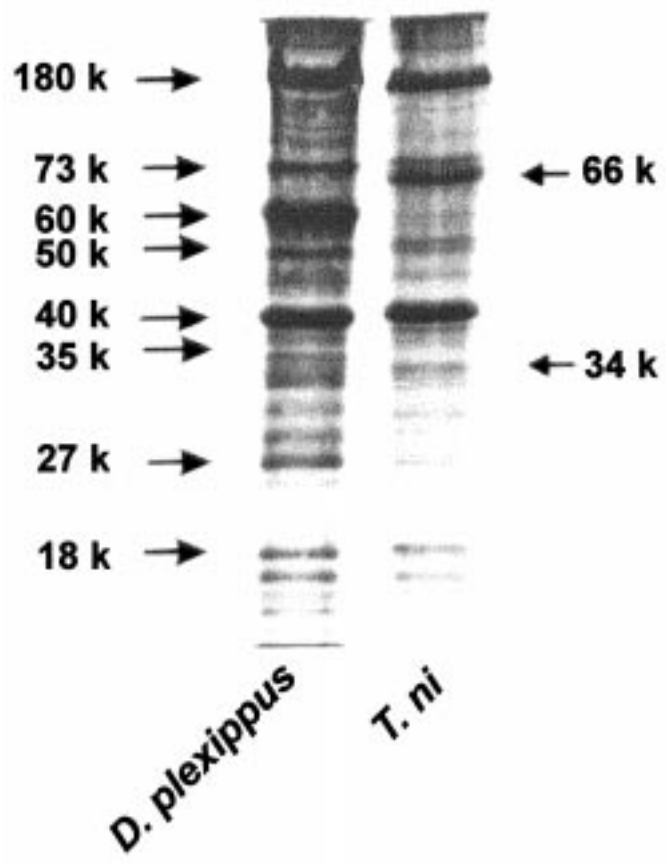

Figure 3. SDS-PAGE of proteins from delipidated insect eggs. Stained with Coomassic Blue.

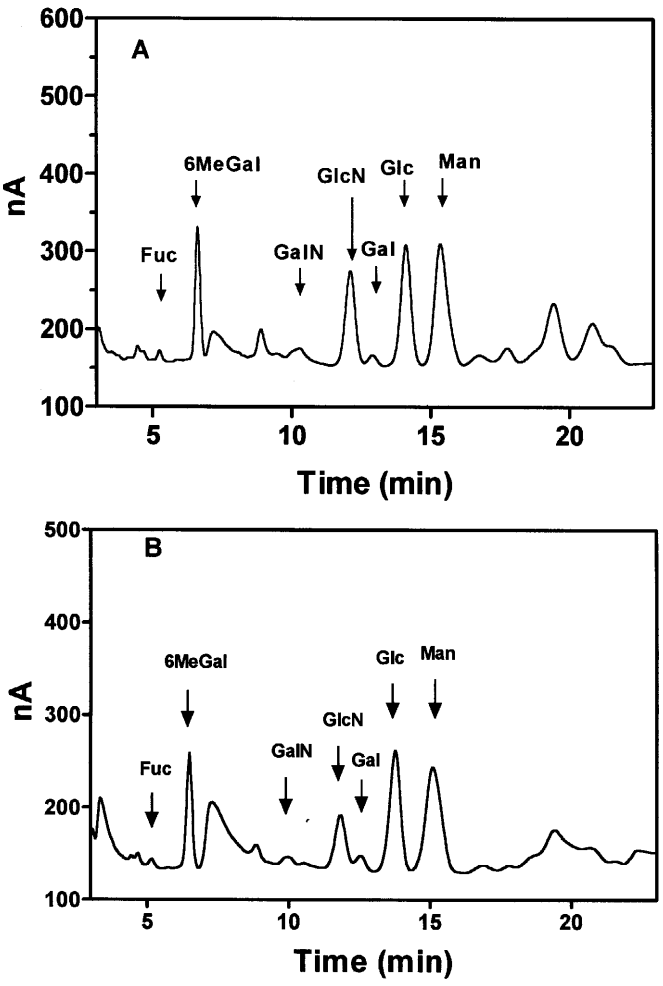

Figure 4. Analysis of sugar compositions of major blotted insect egg glycoproteins with HPAEC/PAD. (A) the 60 kDa protein from *D. plexippus* eggs. (B) the 73 kDa protein from *T. ni* eggs.

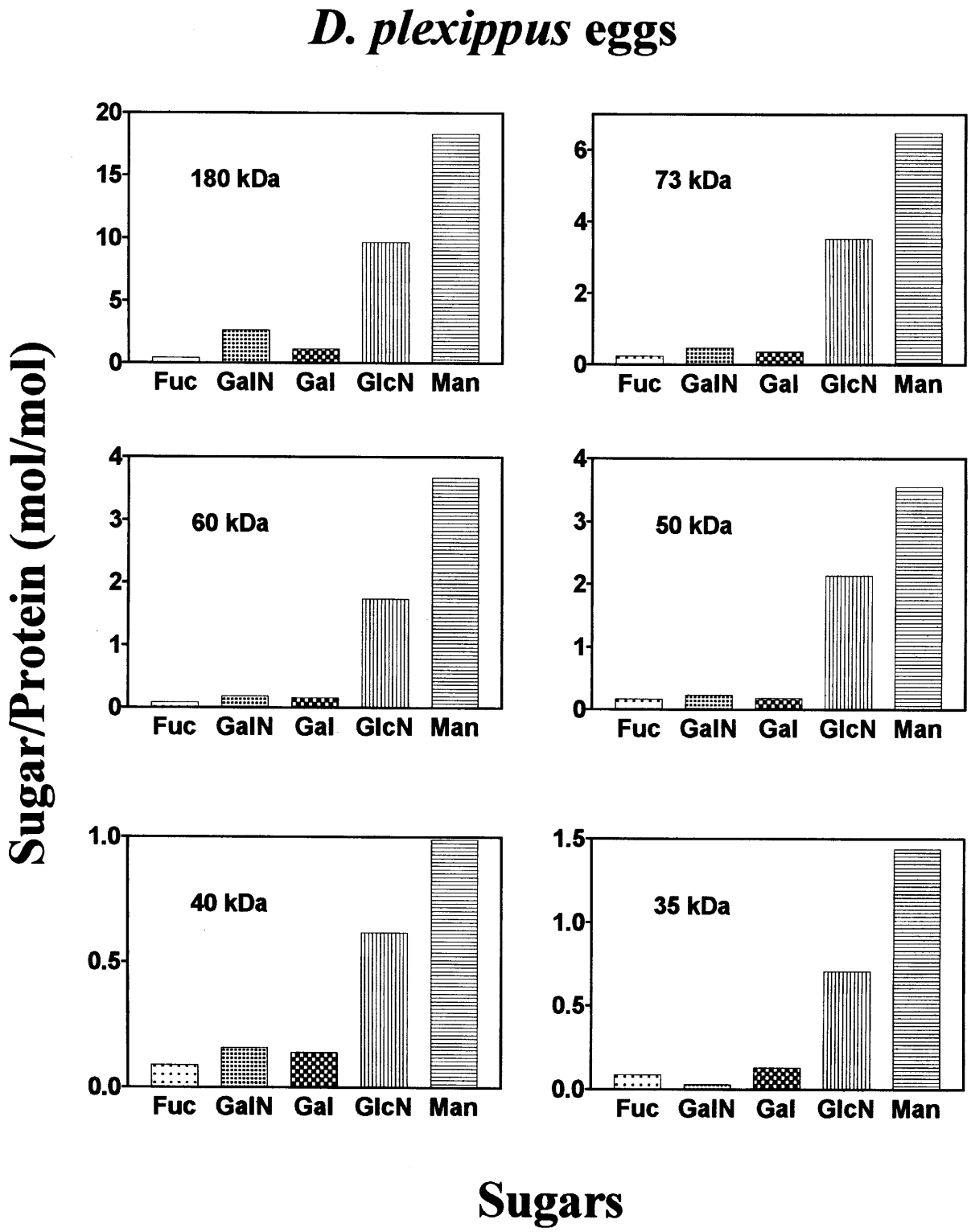

Figure 5. Neutral and amino sugars in the major glycoproteins from *D. plexippus* eggs isolated by SDS-PAGE and blotting.

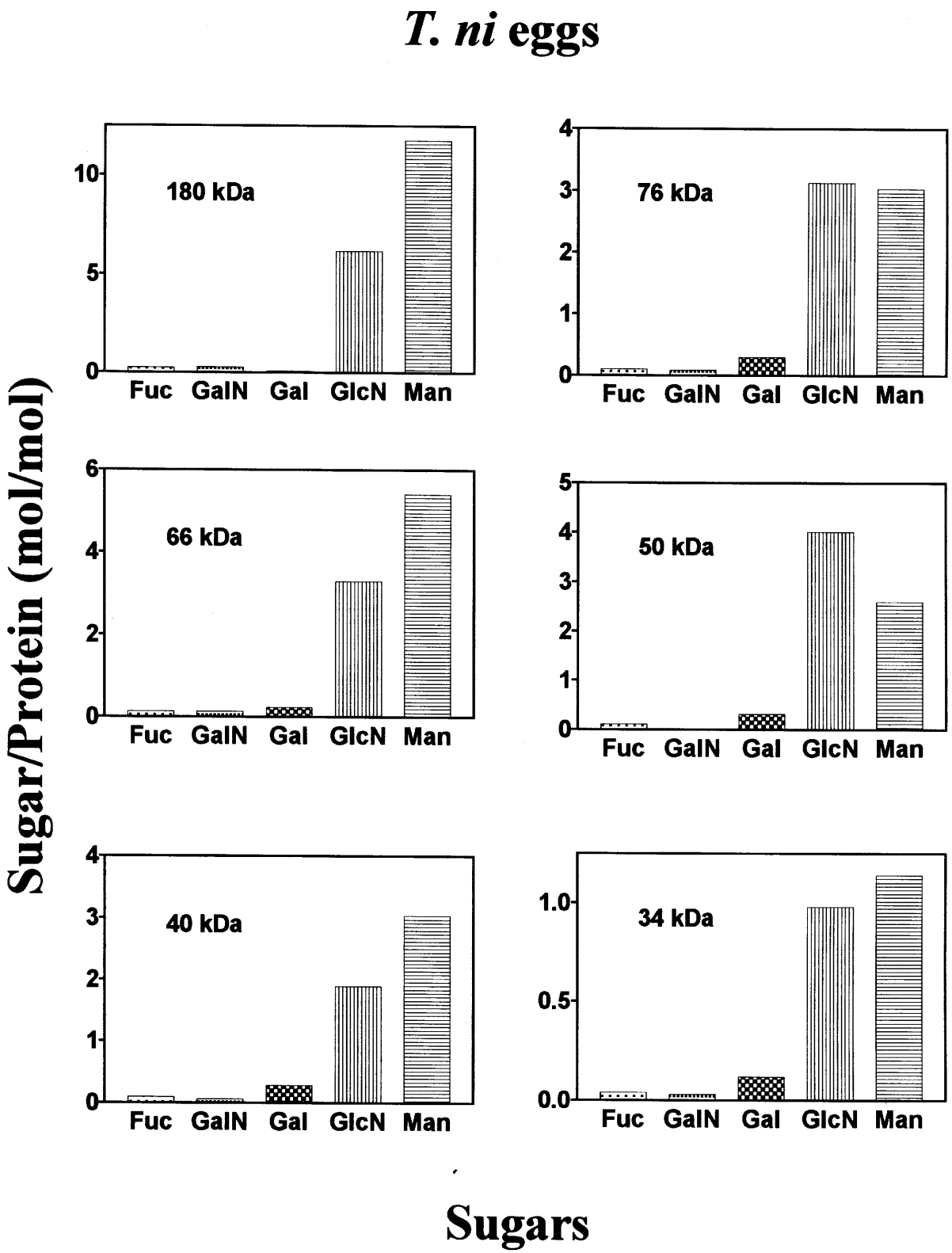

Figure 6. Neutral and amino sugars in *T. ni* eggs.

Table 2. Sugar density of blotted major proteins from *D. plexippus* eggs

Proteins (kDa)	*Carbohydrates/Proteins*[a] (wt %)	*No. of N-glycosylation sites*[b]
180	3.24	4.8
73	2.68	1.8
60	1.7	8.7
50	2.23	1.1
40	0.88	0.31
35	1.2	0.36

[a]Protein was determined by the BCA method.
[b]Assuming two GlcNAc residues per chain.

Table 3. Sugar density of blotted major proteins from *T. ni* eggs

Proteins (kDa)	*Carbohydrates/Protein*[a] *(wt%)*	*No. of N-glycosylation sites*[b]
180	1.80	3.1
76	1.59	1.6
66	2.47	1.7
50	2.62	2.0
40	2.38	0.95
34	1.32	0.49

[a]Determined with the BCA method.
[b]Assuming two GlcNAc residues per chain.

plexippus, and 3.8:2.0 (180 kDa), 1.9:2.0 (76 kDa), 3.3:2.0 (66 kDa), 1.3:2.0 (50 kDa), 3.2:2.0 (40 kDa) and 2.3:2.0 (34 kDa) for the *T. ni* protein bands (Figs. 5 and 6). These ratios are remarkably constant, especially for the *D. plexippus* protein bands, the ratio not exceeding 2.0. In contrast, the Man:GlcN ratios of the crude protein preparations were 5.4:2.0 for *D. plexippus* and 9.4:2.0 for *T. ni*. This again suggests the possible presence in the crude preparations of non-glycoprotein material that contains Man, and points out the advantage of the blotting method for more accurate assessment of glycan structure on glycoproteins.

The Man:GlcN ratios of from 3.2 up to 4.2 for *D. plexippus* egg proteins suggests that majority of glycans on these proteins may be of pauci-Man type. The possible presence of small amounts of Gal and Fuc may indicate the existence of some hybrid structures. The overall picture is quite similar for *T. ni* eggs, except that the Man:GlcN ratio in some protein samples are lower, which might mean larger amounts of hybrid-type structures, especially since the higher amounts of GlcN are often accompanied by higher amounts of Gal (Fig. 6).

In mammalian species, the presence of GalN on glycoproteins often correlates to the O-glycan structures. However, in some rare occasions, GalN can be present as a capping group on N-glycan structures. A similar situation can be envisioned for insect glycoproteins. In fact, the presence of GalNAcβ4GlcNAc in an insect protein has been documented (Kubelka et al., 1993).

Neu5Ac, although possibly present in minute amounts in the crude protein preparations of eggs from both species, could not be detected in any of the blotted protein bands. Based on the detection limit of the DMB method, we would have detected as low as 0.035 mol Neu5Ac per mol protein. It is most likely, therefore, that any Neu5Ac detected in the crude protein preparations arises from some non-glycoprotein sources or from those small glycoproteins which were not transferred or trapped on PVDF membrane.

In conclusion, analysis of carbohydrate of *D. plexippus* and *T. ni* egg proteins showed that the majority of proteins are glycoproteins and as expected, Man and GlcN are the predominant components. The ratio of Man:GlcN suggests that most of glycans on the glycoproteins are pauci-Man type N-glycans. Small amounts of peaks corresponding in migration to Gal, GalN and Fuc are consistently present, suggesting that a small portion of the N-glycans may be of hybrid-type.

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References

1 Davidson DJ, Castellino FJ (1991a) Asparagine-linked oligosaccharide processing in lepidopteran insect cells. Temporal dependence of the nature of the oligosaccharides assembled on asparagine-289 of recombinant human plasminogen produced in baculovirus vector infected *Spodoptera frugiperda* (IPLB-SF-21 AE) cells. *Biochemistry* **30:** 6167–74.

2 Davidson DJ, Castellino FJ (1991b) Structures of the asparagine-289-linked oligosaccharides assembled on recombinant human plasminogen expressed in a *Mamestra brassicae* cell line (IZD-MB0503). *Biochemistry* **30:** 6689–96.

3 Davidson DJ, Fraser MJ, Castellino FJ (1990) Oligosaccharide processing in the expression of human plasminogen cDNA by lepidopteran insect (*Spodoptera fugiperda*) cells. *Biochemistry* **29:** 5584–90.

4 Davis TR, Wood HA (1995) Intrinsic glycosylation potentials of insect cell cultures and insect larvae. *In Vitro Cell. Dev. Biol.* **31:** 659–63.

5 Fan J-Q, Kondo A, Kato I, Lee YC (1994a) High performance liquid chromatography of glycopeptides and oligosaccharides on graphitized carbon columns. *Anal. Biochem.* **219:** 224–9.

6 Fan J-Q, Namiki Y, Matsuoka K, Lee YC (1994b) Comparison of acid hydrolytic conditions for Asn-linked oligosaccharides. *Anal. Biochem.* **219:** 375–8.

7 Hara S, Yamaguchi M, Takemori Y, Furuhata K, Ogura H, M. N (1989) Determination of mono-O-acetylated N-acetylneuraminic acids in human and rat sera by fluorometric high-performance liquid chromatography. *Anal. Biochem.* **179:** 162–6.

8 Hard K, Van Doorn JM, Thomas-Oates JE, Kamerling JP, Van der Horst DJ (1993) Structure of the Asn-linked oligosaccharides of apolipophorin III from the insect *Locusta migratoria*. Carbohydrate-linked 2-aminoethylphosphonate as a constituent of a glycoprotein. *Biochemistry* **32:** 766–75.

9 Honda S, Akao E, Suzuki S, Okuda M, Kakehi K, Nakamura J (1989) High-performance liquid chromatography of reducing carbohydrates as strongly ultraviolet-absorbing and electrochemically sensitive 1-phenyl-3-methyl-5-pyrazolone derivatives. *Anal. Biochem.* **180:** 351–7.

10 Hsu TA, Watson S, Eiden JJ, Betenbaugh M (1996) Rescue of immunoglobulins from insolubility is facilitated by PDI in the baculovirus expression system. *Prot. Expression and Purification* **7:** 281–8.

11 Hughes PR, Radke CD, Renwick JA (1993) A simple, low-input method for continuous laboratory rearing of the monarch butterfly (*Lepidoptera: danaidea*) for research. *Amer. Entomologist* **39:** 109–11.

12 Hughes PR, Van-Beek NAM, Wood HA (1986) A modified droplet feeding method for rapid assay of *Bacillus thuringiensis* and baculoviruses in noctoid larvae. *J. Invertebr. Pathol.* **48:** 187–92.

13 Kubelka V, Altman F, Kornfeld G, März L (1994) Structure of the *N*-linked oligosaccharides of the membrane glycoproteins from three *Lepidopteran* cell Enes (Sf-21, IZD-Mb-0503, Bm-N). *Arch. Biochem. Biophys.* **308:** 148–57.

14 Kubelka V, Altmann F, Staudacher E, Tretter V, März L, Hard K, Kamerling JP, Vliegenthart JFG (1993) Primary structures of the *N*-linked carbohydrate chains from honeybee venom phospholipase A_2. *Eur. J. Biochem.* **213:** 1193–204.

15 Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–5.

16 Lucnow (1994) Protein production and processing from baculovirus expression vectors. In *Baculovirus expression systems and biopesticides* (Schuler ML, Wood HA, Granados RR, Hammer DA eds), pp 51–90. Wiley-Liss, Inc.: New York, NY.

17 März L, Altman F, Staudacher E, Kubelka V (1995) Protein glycosylation in insects. In *Glycoproteins* (Montreuil J, Schachter H, Vliegenthart H eds) vol 3, pp 543–63. Elsevier, B.V.: Amsterdam, The Netherlands.

18 McKelvy JF, Lee YC (1969) Microheterogeneity of the carbohydrate group of *Aspergillus oryzae* α-amylase. *Arch. Biochem. Biophys.* **132:** 99–110.

19 Ogonah OW, Freedman RB, Jenkins N, Patel K, Rooney BC (1996) Isolation and characterization of an insect cell line able to perform complex *N*-linked glycosylation on recombinant proteins. *Biotechnol.* **14:** 197–202.

20 Staudacher E, Kubelka V, März L (1992) Distinct *N*-glycan fucosylation potentials of three *Lepidopteran* cell lines. *Eur. J. Biochem.* **207:** 987–93.

21 Weiss JB, Magnani JL, Strand M (1986) Identification of *Schistosoma mansoni* glycolipids that share immunogenic carbohydrate epitopes with glycoproteins. *J. Immunol.* **136:** 4275–82.

22 Weitzhandler M, Hardy M, Co MS, Avdalovic N (1994) Analysis of carbohydrates on IgG preparations. *J. Pharm. Sci.* **83:** 1670–5.

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