Mucin-type glycoprotein from *Drosophila melanogaster* embryonic cells: characterization of carbohydrate component

A.A. Kramerov[a,*], N.P. Arbatsky[b], Y.M. Rozovsky[a], E.A. Mikhaleva[a], O.O. Polesskaya[a], V.A. Gvozdev[a], V.N. Shibaev[b]

[a]*Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq., 123182 Moscow, Russian Federation*
[b]*N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 117913 Moscow, Russian Federation*

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Abstract **A secreted glycoprotein (GP) with apparent molecular mass of 90 kDa produced by cultured embryonic cells of *Drosophila melanogaster* was isolated and partially characterized. GP is enriched by Ser + Thr and Pro residues that constitute up to 30% of the total number of amino acids. An abundant carbohydrate moiety (40% of molecular mass) is mainly represented by vertebrate mucin-type O-linked disaccharide units Gal(β1–3)-GalNAc, occupying about a half of the total number of Ser + Thr residues and rendering the GP molecule high resistance to protease action. A few of *N*-glycans are also present in GP. These characteristics allow to consider the *Drosophila* GP (termed 'mucin-D') as a first representative of invertebrate mucin-type glycoproteins.**

Key words: *O*-Glycan; Mucin glycoprotein; Cultured cell; *Drosophila*

1. Introduction

Important roles for glycoproteins in specifying various biological functions are now well documented for vertebrates (for reviews see [1,2]) while insect glycoproteins remain poorly investigated. The data available at present mainly concern glycoproteins having N-linked oligomannose chains [3–8], and show some similarity between the mechanism of *N*-glycosylation of proteins in insects and vertebrates. Insect *O*-glycoproteins are much less characterized, their oligosaccharide structure being described only for a couple of objects [9–11] while a growing body of evidence for important roles played by vertebrate *O*-glycoproteins, particularly of the mucin family, in cellular communication, differentiation and carcinogenesis, has recently been accumulated (for review see [12,13]). However, mucin-type glycoproteins have not been previously found in invertebrates and biochemical and cellular characterization of *O*-glycosylation and its possible biological functions in insect cells remain to be elucidated.

A glycoprotein with molecular mass of about 90 kDa, a major fraction of [^{3}H]*N*-acetylglucosamine-labelled glycoproteins synthesized in cultured embryonic cells of *Drosophila melanogaster* was shown to be a target for insect moulting hormone that regulates its biosynthesis in different cell lines [14]. Using specific antibodies this glycoprotein was immunohistochemically localized in a variety of *Drosophila* tissues during the development [15]. In some cases it was characterized as a component of the extracellular matrix secreted by *Drosophila* cells in a stage- and tissue-specific manner [15]. The goal of the present study was to elucidate the biochemical nature of this glycoprotein, and, at the first step, to characterize its carbohydrate moiety. The data obtained allow us to consider this glycoprotein as a first member of the mucin-type family not yet described in invertebrates.

*Corresponding author. Fax: (7) (095) 196 02 21.

2. Materials and methods

2.1. Cell cultures

Established cell line 67j25D [16] of *D. melanogaster*, was cultured either on Schneider medium (Gibco) or C-46 [17] in the presence of 5% fetal calf serum (FCS) (Gibco) as described elsewhere [18]. Cells were replated on fresh medium after every 7 days on reaching a concentration of approx. 10^7 cell/ml. Glycoproteins were detected by 2-day incubation of cultured insect cells with [^{3}H]GlcNAc (Amersham) at a concentration of 0.02–0.04 mCi/ml. An inhibitor of *N*-glycosylation, tunicamycin (Calbiochem) was added to the culture medium at a concentration of 0.001 mg/ml 4–6 h prior to introduction of the labelled precursor.

2.2. Isolation and purification of glycoprotein from cultured cells

To isolate GP a modification of the previously described [19] purification procedure was used. An additional step in the purification scheme included electroblotting of a gel after preparative gel-electrophoresis of the partially purified GP preparation. An unusually low efficiency of electrotransfer of GP from the gel to a membrane, as compared to contaminating proteins, resulted in a high degree of purity of GP material residing within the gel. Subsequent extraction of GP from the Coomassie-stained gel bands with 50 mM Tris-HCl buffer, pH 7.0, 0.1% SDS, yielded pure GP of either 90 kDa (form A) or 80 kDa (form B) molecular masses (see section 3). The isolated GP was dialysed against water and lyophylized yielding glycoprotein preparation with a recovery of about 30% as revealed by immunoblot titration of GP contents in crude extract. Protein determination was performed according to the method of Bradford [20] using Protein Assay Kit (Bio-Rad).

2.3. Autoradiographic and Western blotting detection of glycoprotein

Cells were washed twice with saline solution C-15 [18], lysed in Laemmli buffer [21] and labelled glycoproteins were separated by sodium dodecyl sulfate (SDS) gel electrophoresis in 10%-polyacrylamide gel [21] or in 10–15% gradient Phast-gels (Pharmacia). After fixation in 10% trichloroacetic acid and staining with 0.1% solution of Coomassie blue R-250 gels were impregnated with 2 M sodium salicylate [22], dried and exposed with Kodak XR-like film at −60°C. For Western blot detection proteins were electrophoretically transferred from the gel in 10 mM Na-borate buffer, pH 9.2 for 3 h at 0.4 A onto nitrocellulose filters BA 85 (Schleicher and Schuell) [23]. Filters were treated for 2 h at 25°C in 0.05% Tween 20 in TBS (TTBS) to decrease the non-specificity of immunostaining and then incubated for 1 h at 25°C with specific polyclonal [19] or monoclonal [24] antibodies diluted 1:500 or 1:2000, respectively, in TTBS with 5% FCS. After washing 3 times in TBS the filters were treated for 1 h at 25°C with secondary antibodies conjugated with alkaline phosphatase (Sigma) in 1:1000 dilution in TTBS with 5% FCS. The washed filters were stained for 15 min at 25°C with Br,Cl-indolylphosphate (0.075 mg/ml) and nitro blue tetrazolium (0.05 mg/ml) in 50 mM Tris-HCl, pH 9.5. For lectin staining, GP filters

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after electrophoretic transfer were incubated 1 h with biotinylated lectin solutions (0.01 mg/ml) in TBS with 1 mM $CaCl_2$, $MgCl_2$ and $MnCl_2$. After washing with TBS the filters were treated with the solution of horseradish peroxidase conjugated to streptavidin (Sigma) in 1:500 dilution. Specific staining was developed after addition of 1 mg/ml solution of diamine benzidine (Sigma) and 0.03% H_2O_2 in 0.05 M Tris-HCl, pH 7.5.

2.4. N-Glycanase digestion of glycoprotein

Glycoprotein (0.05 mg) was digested either with 50 mU endoglycosidase F (endo-β-*N*-acetylglucosaminidase F, EC 3.2.1.96) in 0.01 M Na-acetate, pH 4.5, or 200 mU *N*-glycosidase F (glycopeptide-*N*-glycosidase F, EC 3.2.2.18) in 0.1 M Na-phosphate buffer, pH 7.0.

2.5. Release of oligosaccharides by O-glycanase treatment

For splitting off O-linked carbohydrate chains in GP *O*-glycanase (endo-α-*N*-acetyl-D-galactosaminidase, E.C.3.2.1.97, Boehringer) was used. The purified GP (10 μg) was dissolved in 0.1 ml of 50 mM Na-phosphate buffer, pH 7.0, containing 0.5% Triton X-100 and 0.05% SDS, and 1 mU of the enzyme solution (10 U/mg) was added. After incubation at 37°C for 2–8 h, the digestion products were detected after gel electrophoresis either by autoradiography or immunoblot staining.

2.6. Release of oligosaccharides by alkaline elimination [25]

The solution of GP (0.1 ml, 3.5×10^5 counts/min) was mixed with 0.1 M NaOH/2M $NaBH_4$ (0.1 ml) and incubated at 45°C for 8 h and then at 20°C for 16 h. Excess of $NaBH_4$ was destroyed by addition of acetic acid. The reaction mixture was applied onto a 1.5-ml Dowex 50 × 2 (H^+) column that was washed with 5 ml of water to elute the oligosaccharides and with 5 ml of 1 M NH_4OH to elute glycopeptides. The oligosaccharide fraction was coevaporated several times with CH_3OH to remove borate. The radioactivities of oligosaccharide and glycopeptide fractions were determined.

2.7. Chromatography of oligosaccharides

The oligosaccharide mixtures were chromatographed on a Sephadex G-50 column (1.5 × 20 cm) or a TSK-HW40 column (0.8 × 53 cm) calibrated with the following standard mono- and oligosaccharides: GalNAc-ol (**1**), Gal(β1–3)GalNAc-ol (**2**) [26], GlcNAc(α1–4)Gal(β1–3)GalNAc-ol (**3**) [26], Fuc(α1–2)Gal(β1–3)[GlcNAc(α1–4)Gal(β1–4)GlcNAc(β1–6)]GalNAc-ol (**4**) [27]. The oligosaccharides were eluted with water and detected either by measuring UV adsorbtion at 200 nm or the radioactivity of the fractions. Fractions containing the desired material were pooled (see section 3), dried, and separated by chromatography on a 4.6 × 250 mm Ultrasphere-C18 column (Altex, USA) calibrated with the standard mono- and oligosaccharides.

2.8. Quantitative analysis

Carbohydrates were analyzed on a Biotronik LC-2000 analyzer (Germany). For determination of neutral and amino sugars GP was hydrolized with 3 M CF_3COOH (100°C, 6 h) and with 4 M HCl (100°C, 16 h), respectively. The amino acid composition of the GP was assessed after hydrolysis of the protein in the mixture of CF_3COOH and conc. HCl in 1:2 (v/v) ratio at 148°C for 1 h. The hydrolysate was analysed on 835 Hitachi Amino Acid Analyzer.

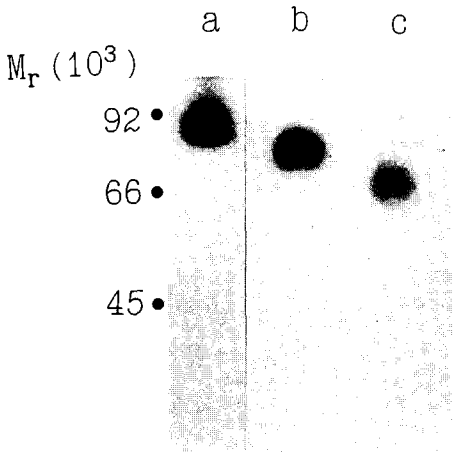

Fig. 1. Polyacrylamide gel electrophoresis of *Drosophila* glycoprotein labelled with [^{3}H]GlcNAc (a) and digested with *O*-glycanase for 2 h (b) and 8 h (c). Figures to the left indicate the molecular masses of the protein markers: phosphorylase b, bovine serum albumin and ovalbumin (92, 66 and 45 kDa, respectively).

Table 1
Amino acid and carbohydrate composition of GP (number of residues per 100 amino acids)

Amino acids				Carbohydrates	
Asp + Asn	7.5	Met	1.1	GlcNAc	1.4
Thr	11.5	Ile	2.2	GalNAc	12.2
Ser	10.8	Leu	5.5	Gal	9.5
Glu + Gln	10.5	Tyr	1.4	Man	2.0
Pro	7.3	Phe	3.5	Fuc	0.5
Gly	15.0	Lys	4.6	Xyl	1.0
Ala	8.4	His	0.9	Glc	2.2
Cys	1.5	Arg	3.3	Rha	traces
Val	4.0			Rib	traces

3. Results

3.1. Amino acid and carbohydrate composition of GP

The isolated GP labelled by 3H-GlcNAc has an apparent molecular mass of 90 kDa as revealed by SDS-gel electrophoresis (Fig. 1); its amino acid and carbohydrate composition is presented in Table 1. A distinctive feature of GP is a high content of Ser, Thr and Pro, that are typical for mucin-like *O*-glycosylated proteins. These amino acids along with Gly contribute to a protein about 45% of all residues.

The monosaccharide composition is also quite typical for vertebrate *O*-glycoproteins: GalNAc and Gal are the major constituents, while Man and GlcNAc - the minor ones (Table 1). The finding of monosaccharides unusual for animal glycoproteins (Xyl, Rha, Rib, Glc) can probably be assigned to some contamination in the GP sample. The molar amounts of Ser, Thr and GalNAc in the GP molecule are nearly equal; assuming a single GalNAc per carbohydrate chain, it means that about one half of the total number of hydroxyaminoacids are *O*-glycosidically linked to oligosaccharides. Besides, the quantity of Man and GlcNAc residues in GP sample allows us to suggest the presence of some *N*-glycans in the GP molecule.

3.2. Analysis of oligosaccharide fractions obtained by treatment of GP with O-glycanase and alkaline $NaBH_4$

Digestion of the GlcNAc-labelled GP by *O*-glycanase resulted in decreasing of its molecular mass to 65 kDa and in splitting off the majority of the labelled sugar component (Fig. 1). After gel-filtration of the digestion products on Sephadex G-50 an oligosaccharide fraction comprising approx. 70% of the radioactivity was isolated (data not shown). Monosaccharide composition of this fraction proved to be quite similar with respect to relative Gal and GalNAc contents, as compared to that of the whole GP (Table 2). This oligosaccharide mixture was further reduced by $NaBH_4$, deionized on cation-exchanger and analysed by gel-chromatography on TSK HW-40 column. The most part of carbohydrates were revovered in the fraction A which coeluted with the standard disaccharide **2** [Gal(β1–3)GalNAc-ol] (Fig. 2a).

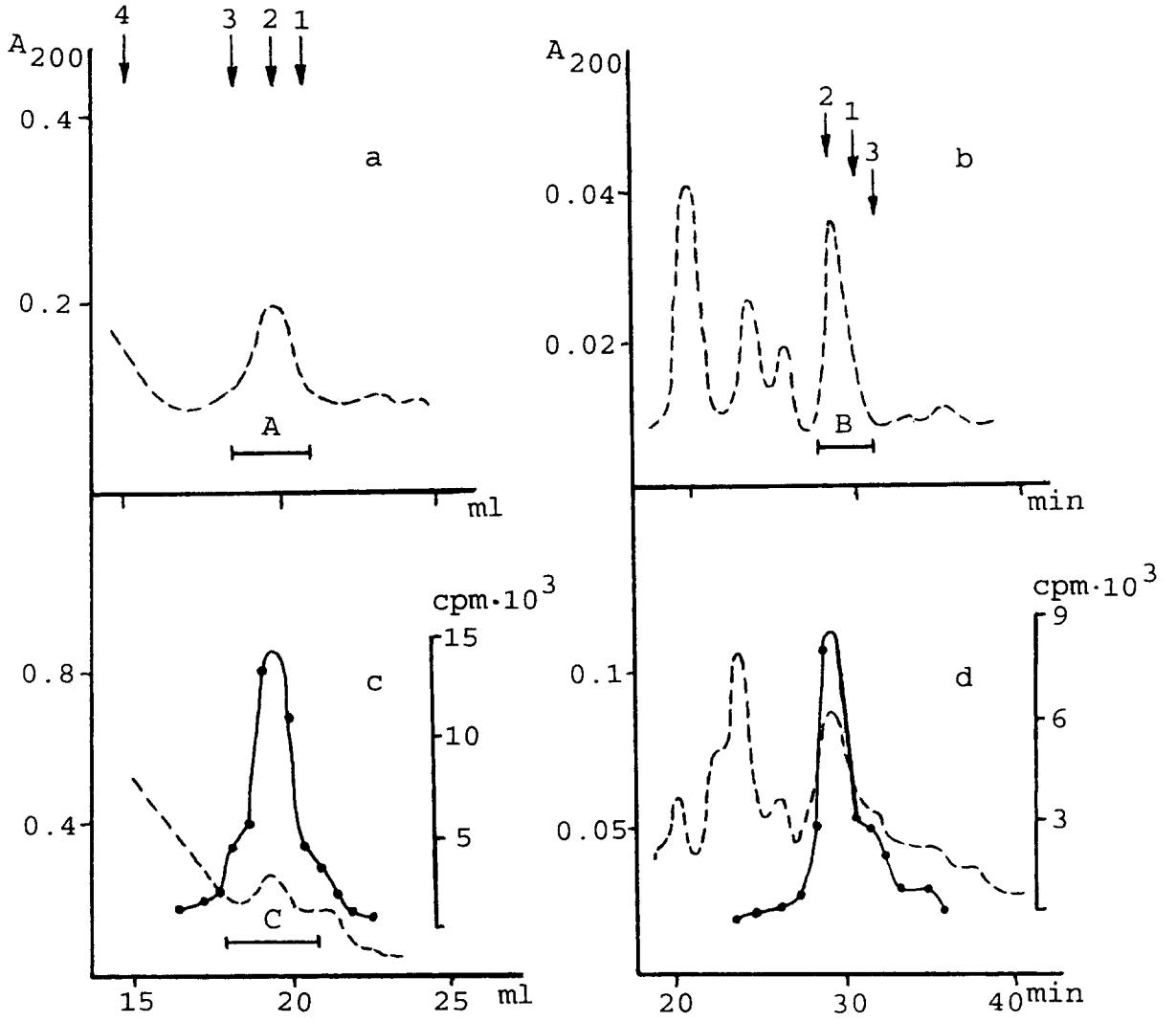

Fig. 2. Isolation of oligosaccharides after digestion of GP with *O*-glycanase (a,b) and after treatment of GP with $NaOH/NaBH_4$ (c,d). Fractionation on TSK HW-40 (a,c) and on Ultrasphere-C18 (b,d) columns. Oligosaccharides were detected by measuring UV adsorption and ^3H-label content (dashed and continuous lines, respectively). GalNAc-ol (**1**), oligosaccharides Gal(β1–3)GalNAc-ol (**2**), GlcNAc(α1–4)Gal(β1–3)GalNAc-ol (**3**), Fuc(α1–2)Gal(β1–3)[GlcNAc(α1–4)Gal(β1–4)GlcNAc(β1–6)]Gal-NAc-ol (**4**) obtained earlier [26,27] were used to calibrate the column.

Analysis of the fraction A using Ultrasphere-C18 column, revealed that practically all the radioactivity (not shown) is recovered in the oligosaccharide (fraction B) coinciding with the standard disaccharide **2** (Fig. 2b). Nearly equimolar ratio of Gal and GalNAc-ol was revealed after acid hydrolysis of fractions A and B (Table 2). The finding that all GalNAc turned into the reduced form (GalNAc-ol) indicates that this monosaccharide is completely involved in formation of *O*-glycosidic links with hydroxyamino acids. Fraction A included also a significant amount of GlcN (Table 2), but no distinct GlcN-containing component was revealed after chromatography on Ultrasphere-C18. Thus, Gal(β1–3)GalNAc disaccharide is the only oligosaccharide identified after *O*-glycanase digestion of GP.

Table 2
Carbohydrate contents (nmoles) of the glycoprotein and oligosaccharide fractions obtained after *O*-glycanase digestion

Sugar	Fraction			
	Glycoprotein	Oligosaccharide fraction	Reduced oligosaccharide	
			Fraction A	Fraction B
GlcN	18	24	21	traces
GlcN-ol	0	0	5	traces
GalN	170	108	traces	0
GalN-ol	0	0	45	15
Gal	130	82	53	14
Man	30	8	3	traces
Xyl	14	5	traces	traces
Fuc	8	4	0	0

While *O*-glycanase is able to split only a certain kind of sugar chains, we used mild alkaline elimination of *O*-glycosidically linked chains in the presence of $NaBH_4$ [25] which allows to obtain all types of *O*-glycans. After fractionation of thus obtained oligosaccharides by gel chromatography on TSK HW-40 the radioactivity was detected mainly in a fraction corresponding to disaccharide 2 (fraction C, Fig. 2c) which was further

analyzed on Ultrasphere-C18 column. Practically all the radioactivity was recovered in the disaccharide 2 fraction though a small amount of the label could be detected in the shoulders of the main peak corresponding to mono- and trisaccharide (Fig. 2d). Thus, the main chemically cleavable GP's carbohydrate chains proved to be the disaccharide Gal(β1–3)GalNAc comprising the core-1 fragment of various *O*-glycans, and, especially, those of vertebrate mucin-type glycoproteins.

3.3. *Variation of glycan structure of* Drosophila *glycoprotein*

Two different forms of the glycoprotein under study were clearly revealed in a subline of *Drosophila* cells harvested on another culture medium, C-46 [17]. In these conditions the cells produce, along with the described above 90-kDa GP (GP-A), a novel form (GP-B) with molecular mass of 80 kDa. Both forms of GP are recognized by polyclonal antibodies against GP-A (Fig. 3a). GP-B, as opposed to GP-A, is not susceptible to the action of *O*-glycanase (Fig. 3b) which, due to splitting off the disaccharide units from GP-A, causes a dramatic shift of its mol. mass from 90 kDa to 65 kDa (Figs. 1 and 3b). GP-B is not recognized by monoclonal antibodies to GP-A (Fig. 3c) which are specific for the O-linked Gal(β1–3)GalNAc disaccharide (data not shown) representing the main type of sugar chains in GP-A. To elucidate the nature of the observed differences between the GP forms, biotinylated lectins were used as specific tools for probing carbohydrate structure. GP-B proved to be non-reactive with PNA (Galβ1–3GalNAc-specific) and poorly recognisable by RCA-2 (Gal-specific), while GP-A showed high reactivity to these lectins (Fig. 3d,e). Noticeable and similar recognition of two forms of GP was detected with RCA-1 (GalNAc>>Gal) and ConA (Man>>Glc) (Fig. 3f,g). These data may probably indicate that, in contrast to GP-A carrying Galβ1–3GalNAc as a major type of sugar chains, GP-B contains mainly unsubstituted single O-linked GalNAc residues. This proposal was corroborated by comparative analysis of monosaccharide content of the purified GP-B preparation. Really, the molar ratio of Gal to GalNAc in GP-B was about 8 times lower than that in GP-A (see Table 3) and analysis of the products formed after alkaline $NaBH_4$ degradation allowed to identify GalNAc as a predominant O-linked sugar in GP-B (data not shown). This difference in sugar content might explain the observed molecular mass shift between the two GP forms.

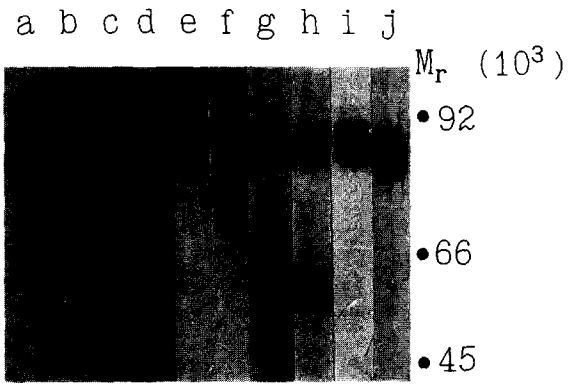

Fig. 3. Western blot analysis of *Drosophila* GP glycoforms (a–g) and inhibition of *N*-glycan synthesis by tunicamycin (h–j). Filters were stained by polyclonal (a,b), monoclonal (c) antibodies, PNA (d), RCA-2 (e), RCA-1 (f) and Con A (g,h). Lane b represents GP after *O*-glycanase treatment. Control (g,i) and tunicamycin-treated (h,j) cells; Con A staining (g,h) and autoradiographic detection of [^{3}H]GlcNAc labelled GP (i,j).

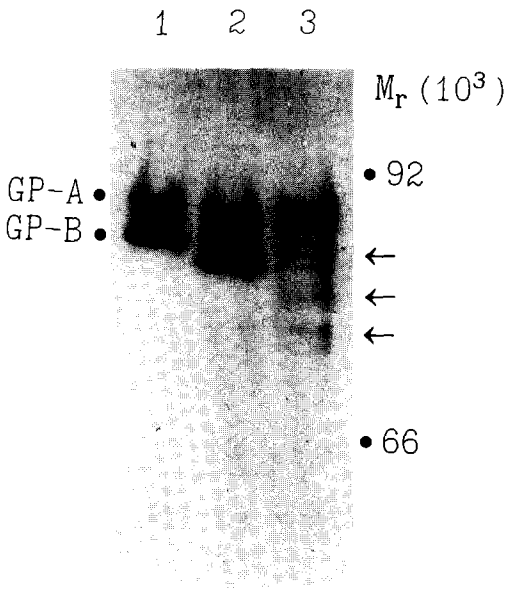

Fig. 4. Western blot analysis of GP glycoforms treated with *N*-glycanases, as revealed by polyclonal antibodies staining: (1) control, (2) Endoglycosydase F, (3) Glycopeptidase F.

As indicated by Con A reactivity and sugar composition both forms contain Man and GlcNAc residues, presumably as constituents of *N*-glycans. To demonstrate this directly we used an inhibitor of *N*-glycosylation, tunicamycin, and *N*-glycanases. Treatment of the cultured cells with tunicamycin resulted in accumulation of the labelled derivatives of both GP forms with decreased molecular mass values (Fig. 3i,j), that were not reactive to Con A which, at the same time, stained the performed GP-A and GP-B produced before administration of the drug and still present in the cells (Fig. 3g,h). Two forms of GP proved to be susceptible to the action of endo-β-*N*-acetylglucosaminidase F (EndoF, cleavage site GlcNAc-\-GlcNAc-Asn) which yielded bands of lower apparent molecular mass values (Fig. 4, lane 2). Glycopeptidase F (GlyF, cleavage site GlcNAc-\-Asn) treatment, while causing no effect on GP-A, resulted in elimination of GP-B band, yielding 3 faint bands, which represented descrete partially *N*-deglycosylated GP-B forms carrying different numbers of the N-linked chains (Fig.4, lane 3). Pretreatment of GP-A with *O*-glycanase resulted in its sensitivity to the action of GlyF (not shown), thus indicating an apparent similarity between the glycoforms in their *N*-glycosylation pattern.

4. Discussion

The results of sugar and aminoacid analysis of GP and identification of the major type of its oligosaccharides allow us to propose the GP structure. Based on the apparent molecular mass of the polypeptide chain (about 60 kDa), it is estimated to be composed of approx. 500 amino acid residues and is enriched with Thr, Ser and Pro which amount to 30% of the amino acid residues. Roughly every fifth amino acid residue is either Thr or Ser, and more than a half of these carry O-linked disaccharide units. There is, on average, one carbohydrate

Table 3
Carbohydrate contents of the glycoprotein glycoforms (mole/mole of GP)

Sugar	GP-A	GP-B
GlcN	8	6
GalN	51	67
Gal	29	5
Man	10	14
Xyl	4	2
Fuc	<2	<1
Glc	11	43

chain per 8–10 amino acids. Thus, disaccharide units cover a significant part of the polypeptide and may account for its proteolytic resistance. In this respect *Drosophila* GP, for which we propose the term 'mucin-D', is closely related to vertebrate mucin glycoproteins which contain up to 80% carbohydrate providing the whole mucin molecule both high rigidity and proteolytic stability [13]. However, whereas vertebrate mucins possess heterogeneous sugar side chains (up to 20 residues long), 'mucin-D' contains short homogeneous O-linked carbohydrate component consisting mainly of Gal(β1–3)GalNAc disaccharides. This property is similar to that of the 'antifreeze' glycoprotein from antarctic fish, in which case the carbohydrate moiety is represented solely by the disaccharide Gal(β1–3)GalNAc [28]. Earlier, using different proteases we obtained some evidence for rather even distribution of O-chains on a polypeptide backbone of the 'mucin-D' [29]. The GP was relatively resistent to pronase E (at low enzyme concentrations) and was cleaved to completion by increased concentrations (1–5 mg/ml) with no high molecular weight intermediates formed. This might indicate the absence in 'mucin-D' of carbohydrate chain clusters or long nonglycosylated polypeptide stretches. Additional studies should be carried out to characterize the distribution of the disaccharide moieties on the protein molecule in detail.

Variations in glycosylation pattern caused by different culture conditions, reported for vertebrate cells [30], were revealed also in this work. After transfer to culture medium C-46 *Drosophila* cells switch on the production of a new form of GP (GP-B) as a predominant type with the original one (GP-A) still expressed. Several dozens of disaccharide units constitute major O-glycan chains in GP-A, while similar amount of single GalNAc residues - that of GP-B. The absence of intermediate glycoforms with molecular mass values between 80 kDa and 90 kDa may indicate these two GP forms to be produced by two distinct clones of cells. For the reasons which remain obscure, after transferring to the new medium cells producing GP-B form gain an advantage in proliferation, and partially substitute the other type of cells. We propose that activity of galactosyltransferase, needed for formation of Gal(β1–3)GalNAc units in GP-A, is very low or absent in cells producing GP-B, leading to formation of very short *O*-glycans (O-linked GalNAc). O-linked terminal GalNAc has recently been found in mucin glycoproteins from vertebrate carcinoma cells (so called Tn-antigen), and is currently considered as a transformed cell marker [13].

N-Glycosylation of the two GP forms, probed by tunicamycin treatment and Con A reactivity, seems to be quite similar. An apparent difference in the GlyF-sensitivity of two glycoforms could, probably, be due to different sizes of their *O*-glycan chains rather then to variable structure of *N*-glycans itself. In the case of GP-A the disaccharide may hinder the access of GlyF to its cleavage site (GlcNAc-/-Asn) causing no or lesser effect on the EndoF site (GlcNAc-/-GlcNAc-Asn). Splitting off N-chains from Gp-A by GlyF proceedes successfully only after removal of the majority of O-linked Gal-GalNAc chains by *O*-glycanase. Suggesting that the two clones differ in the activity of a single enzyme, galactosyltransferase, involved specifically in the *O*-glycan elongation reaction, it seems unlikely that they could also differ by some other enzymes of the *N*-glycosylation pathway. Nevertheless, this possibility can not be excluded and has to be further investigated.

Earlier, we demonstrated high level of incorporation of labelled [^{3}H]GlcNAc into the GP (GP-A), 10 to 30 times higher than the incorporation of other sugars [29]. This suggested that the GP has a chitin-like carbohydrate component. However, this study has revealed that the bulk of the radioactivity is recovered in Gal-GalNAc disaccharide. Obviously, [^{3}H]GlcNAc is rapidly converted in *Drosophila* cultured cells into [^{3}H]GalNAc that, finally, is incorporated into the glycoprotein. The possibility of this conversion of sugars [31,32] should be taken into account when studying structure of carbohydrate moiety of glycoproteins using labelled metabolic precursors.

Data on the structure of *O*-glycoproteins from insects is sparse and limited to a few publications which do not concern their amino acid composition. Single O-linked GalNAc residues were found, along with N-linked oligosaccharides, in the major membrane glycoprotein from cultured mosquito cells [9]. The disaccharide Gal-GalNAc was proposed to be present in vitellogenin receptor of locust ovary cells [11]; a conclusion about the type of O-chains involved was made, however, only on the basis of binding of the glycoprotein to the lectin. Thus, the data available indicate that *O*-glycans of insects are comparatively simple in structure and are composed of 1 to 3 monosaccharide residues including, probably, that of sialic acid [11].

The glycoprotein from *Drosophila melanogaster*, 'mucin-D', described in this publication, containing several dozens of mucin-type O-glycans (Galβ1–3GalNAc or GalNAc) and the protein part enriched in Ser and Thr, is the first representative of mucin glycoproteins found in insects. The immunohistochemical localization of mucin-D in ovary cells [16] suggests that, along with the locust vitellogenin receptor [11], it can be considered as a members of the family of insect mucin-type glycoproteins which play some role in egg maturation. An interesting parallel can be drawn with vertebrate mucins which are present in the extracellular coat (the zona pellucida) of mouse oocyte and account for its sperm receptor activities [33].

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