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Differentiation-dependent glycosylation of gp190, an oncofetal crypt cell antigen expressed by Caco-2 cells

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gp190 is a glycoprotein expressed on the cell surface of several human colon carcinoma cells in culture, on epithelial cells of fetal colon, but not on the normal mucosa of adult colon; thus it is referred to as an oncofetal crypt cell antigen. We report the characterisation of *O*-linked glycans carried by gp190 synthesised by [^{3}H]glucosamine-labelled Caco-2 cells at the confluence (undifferentiated cells) and at three weeks of postconfluence (differentiated cells). By using a specific monoclonal antibody, gp190 was isolated and analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The mobility of gp190 from differentiated cells was found to be lower than that from undifferentiated cells, suggesting a more extensive glycosylation process in the former glycoprotein. The major results of the glycan characterisation have been as follows: (i) gp190 carries mainly, if not exclusively, *O*-linked glycans with the core-2 structure; (ii) the elongation with *N*-acetyllactosamine units of the Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAc tetrasaccharide predominates in gp190 synthesised by differentiated cells, whereas the direct α2,3sialylation of the tetrasaccharide is prevalent in gp190 synthesised by undifferentiated cells. The increment in the core-2 β1,6GlcNAc-transferase activity under the Caco-2 differentiation process may be relevant in producing the larger occurrence of polylactosaminoglycans in gp190 from differentiated cells. Since no change in the activity of the α2,3sialyltransferases upon cell differentiation was observed, we suggest that the lower α2,3sialylation in gp190 synthesised by polarised cells might be due to a changed transit-rate through the distal Golgi apparatus.

Keywords: **colon carcinoma cells, *O*-glycosylation, enterocyte differentiation, polylactosamine glycans, α2,3sialylation**

Introduction

The availability of monoclonal antibodies recognising surface antigens of human colon carcinoma cells has remarkably contributed to the knowledge that neoplastic transformation of colonocytes is associated with an altered expression of integral-membrane glycoproteins and mucins [1]. Several epitopes recognised by a panel of these monoclonal antibodies do not appear to be present in normal mucosa of adult colon but are present in epithelial cells from fetal colon as well as in epithelial cells of adult small-intestinal cells, indicating that they represent markers of a specific stage of intestinal cell differentiation [2–4]. These antigens have been referred to as oncofetal crypt cell antigens [4]. By using monoclonal antibodies produced from mice immunised with brush border membranes of human colon adenocarcinoma Caco-2 cells, Quaroni demonstrated that a fucosylated glycoprotein, probably of mucin-type, with an apparent molecular weight of 190 kDa (gp190) is specifically immunoprecipitated from Caco-2 cells [5]. The human colon carcinoma Caco-2 cell line has been extensively used to study the enterocyte differentiation, in that the cells differentiate spontaneously when maintained in culture for 2–3 weeks after the confluence [6]. At that time of postconfluence, Caco-2 cells express in a very high degree some brush border enzymes and acquire the apical/basolateral polarity [7–9].

There is a large body of evidence that neoplastic transformation results in an altered biosynthesis of *O*-glycans [10]. In Caco-2 cells changes in the *O*-glycosylation and in the activity of some glycosyltransferases have been observed during the differentiation process [11–17]. Only in some cases the changes in the activity of glycosyltransferases during the differentiation process have been found to be consistent with the glycosylation of glycoproteins produced by undifferentiated or differentiated cells. Therefore the glycan structure of a single glycoprotein cannot be merely predicted on the basis of the differentiation-dependent expression of the glycosyltransferases.

In this contest, we undertook a study in order to characterise the glycomoiety of gp190 synthesised by Caco-2 cells at the

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confluence (undifferentiated cells) and after three weeks of postconfluence (differentiated cells). Our results indicate that the gp190 caries predominantly *O*-glycans with the core-2 structure. The glycan elongation by *N*-acetyllactosamine units appears to be prevalent in gp190 synthesised by differentiated cells, whereas the direct addition of α2,3-linked sialic acid to Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAc predominates in the glycoprotein from poorly differentiated cells.

Materials and methods

Coupling of anti-gp190 monoclonal antibody to CNBr-Sepharose 4B

Monoclonal antibody (designated 190/1/266) specific for the polypeptide portion of gp190 was kindly provided by Dr. A. Quaroni. The ascitic fluid (0.5 ml) was brought to 50% saturation of ammonium sulphate and the precipitated monoclonal antibodies, after exhaustive dialysis against 0.1 M $NaHCO_3$, were coupled with 0.2 g of CNBr-Sepharose 4B (Pharmacia) as recommended by the manufacturers. The monoclonal antibodies were coupled to the gel with 90% efficiency.

Cell culture and radioactive labelling

Caco-2 cells obtained from Dr. G. Bises (Istituto Nazionale della Nutrizione, Rome, Italy) were used between passages 50 and 58. Cells were maintained in DMEM supplemented with 20% fetal calf serum and refed three times a week. Cells were subcultured when they were 80% confluent. The cells were seeded at a density of $8 \times 10^4/cm^2$ in 25 cm^2 flasks, usually the confluence was reached after 3–4 days from the seeding. When the cells were maintained in culture for 3 weeks the conditions were as above indicated. For labelling with [^{3}H]glucosamine the normal medium was replaced with a medium containing only 100 mg/litre glucose and 100 μCi/ml of [^{3}H]glucosamine (Amersham, Buckinghamshire, U.K., specific activity 20 Ci/mmol). Labelling was conducted for 24 h.

Isolation of gp190 from labelled Caco-2 cells

At the end of labelling period cell monolayer was washed twice with 20 mM Na-phosphate buffer, pH 7.5, containing 140 mM NaCl (PBS). The cells were scraped off, lysated with PBS containing 1% NP-40, 1% DOC, 0.2 mM TPCK and 0.2 mM TLCK (buffer A) and centrifuged for 1 h at 100 000 *g*. The supernatants were incubated overnight with the monoclonal antibody bound to CNBr-Sepharose 4B beads at 4 °C. At the end of incubation, the beads were washed twice with buffer A and once with 50 mM Tris-HCl pH 7.5 containing 140 mM NaCl. The beads were then suspended in 0.1 M Tris-HCl, pH 8, containing 1% SDS and 5% 2-mercaptoethanol, boiled for 3 min and the soluble portion subjected to SDS-PAGE under reduced conditions on a gradient (6–15%) of polyacrylamide gel followed by fluorography using Amplify (Amersham).

Preparation of [^{3}H]glucosamine-labelled glycopeptides by Pronase digestion

The procedure was as previously described [18]. The areas of gels corresponding to gp190 were excised, cut in small portions and subjected to Pronase digestion. At the end of the digestion (overnight at 60 °C) 1 ml of water was added, the mixture boiled for 10 min and centrifuged. The pellet was again suspended in 1 ml of water and re-treated as above. In the two supernatants about 90% of the radioactivity present in the gel was recovered.

Column chromatography

The chromatography of glycopeptides or reduced glycans on Bio Gel P-4 (400 mesh) was conducted on a column (1 × 75 cm) equilibrated in 0.1 M pyridine/acetate buffer, pH 5. Fractions (1 ml) were collected at a flow rate of 1 ml/h. The Bio-Gel P-4 column had been calibrated with various oligosaccharide markers as previously described [18]. The DEAE-Sephacel chromatography was performed in a column (1 × 12 cm) as detailed elsewhere [19]. Recovery of radioactivity after chromatography on either column was 85–95%.

Mild alkaline borohydride treatment of glycopeptides

Glycopeptides were treated with 1 M $NaBH_4$ in 0.1 M NaOH for 72 h at 37 °C [18]. At the end of treatment, samples were acidified with acetic acid and lyophilised. To remove methyl-borate samples were evaporated four times with methanol/acetic acid and then chromatographed on the Bio-Gel P-4 column.

Glycosidase treatments

Monosialylated reduced oligosaccharides fractionated by DEAE-Sephacel chromatography were digested with NDV neuraminidase (Genzyme Corporation, Cambridge, MA, USA) as described by Paulson et al. [20]. Digestion with endo-β-galactosidase from *Escherichia freundii* (Seikagaku Kogyo, Tokyo) was performed with 100 milliunits in 100 μl of sample in 0.1 M sodium acetate buffer, pH 5.6 for 24 h at 37 °C.

HPLC analysis

A Water apparatus equipped with a Merck LiChrospher 100-NH_2 column (μm 5) was used. For the sialylated reduced glycans the mobile phase was 78:22 acetonitrile: 15 mM KH_2PO_4 at a speed of 2 ml min^{-1} (system A) [21]. The neutral reduced glycans were fractionated as described by Saitoh et al. [22]; the mobile phase was isocratic for 5 min at 90% acetonitrile, followed by a gradient to 60% acetonitrile

in 75 min (system B). Standard oligosaccharide markers were as previously described [18].

Glycosyltransferase assays

Confluent and 3-weeks post-confluent Caco-2 cells were washed three times with cold PBS and homogenised in ice cold water and the homogenate was used as a source of enzyme.

Core-2 β1,6-N-acetylglucosaminyltransferase (β1,6GlcNAc-T)

The incubation mixture and the isolation of GlcNAcβ1,6 (Galβ1,3)GalNAcα1-*O*-benzyl were as described [23].

α2,3Sialyltransferase towards Galβ1,3GalNAc [α2,3(O)ST]

The assay mixture with 2 mM Galβ1,3GalNAcα1-*O*-benzyl as an acceptor was as described by Piller *et al*. [24]. After incubation for 1 h at 37 °C the sialylated acceptor was isolated from the reaction mixture by DEAE-Sephacel chromatography as previously described [19].

α2,3sialyltransferase towards Galβ1,4GlcNAc [α2,3(N)ST]

The assay mixture was as previously described [16] using 0.65 μmol of Galβ1,4GlcNAc as an acceptor. The NeuAcα2,3-Galβ1,4GlcNAc isomer was separated from the reaction mixture by HPLC as described [16].

Analytical methods

ALP activity was determined in cell homogenates by the ALP Kine test (p-nitrophenylphosphate kinetic method) as described by the manufacturer (Sclavo Diagnostic – Milan, Italy). DPP-IV enzyme activity was determined in cell homogenates according to Nagatsu et al. [25] using glycyl-L-proline-4-nitroanilide as substrate. The sucrase activity was assayed in the cell membranes as detailed previously [14]. Proteins were determined by the Lowry method with BSA as standard [26]. The radioactive amino-sugar composition of [^{3}H]glucosamine-labelled glycans was performed as previously detailed [18].

Results

Isolation of gp190 from undifferentiated and differentiated Caco-2 cells

In Caco-2 cells maintained in culture for 3 weeks, the formation of characteristic domes was observed and ALP, DPP-IV and sucrase activities were found to be of several times higher than those detected at the early time of the confluence (Table 1). These results indicate that postconfluent cells were actually well differentiated. Figure 1 shows the SDS-PAGE of [^{3}H]glucosamine labelled gp190 from differentiated and undifferentiated cells. Both gp190s migrated as diffuse bands, typical of heavily glycosylated proteins, but the mobility of gp190 from differentiated cells was slower than that from undifferentiated cells and the intensity of the former band higher than that of the latter. Similar results were obtained in three experiments of cell labelling, strongly suggesting that a higher extent of glycosylation occurs when gp190 is produced by differentiated cells.

Table 1. Brush-border enzyme activities in confluent (undifferentiated) and 3-weeks post-confluent Caco-2 cells (differentiated cells). Activities of sucrase, DPP-IV and ALP were determined as described in the text and expressed as milliunits/mg of proteins. One unit of sucrase and DPP-IV is defined as the activity that hydrolyses 1 μmol of substrate/min at 37 °C ALP activity is expressed in arbitrary units. Values are means ± SD of three independent experiments.

	Activity (mU/mg of protein)	
	Undifferentiated cells	*Differentiated cells*
Sucrase	8 ± 1	35 ± 10
DPP-IV	70 ± 40	383 ± 17
ALP	67 ± 29	370 ± 104

Fractionation of *O*-glycans from [^{3}H]glucosamine-labelled gp190

Although no characterisation of gp190 glycans was performed, it has been suggested that gp190 is a mucin-like glycoprotein highly fucosylated [5]. To identify the oligosaccharide assembly of the two gp190 species, the portions of gel corresponding to the bands of Figure 1 were excised, subjected to Pronase digestion and fractionated by Bio-Gel P-4 filtration. About 90% of [^{3}H]glucosamine-derived radioactivity incorporated

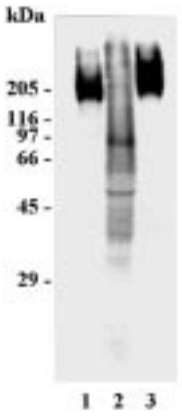

Figure 1. SDS-PAGE of gp190 from undifferentiated and differentiated Caco-2 cells. After 24h of [^{3}H]glucosamine-labelling, cell-lysate was immunoprecipitated from undifferentiated cells (lane 1) and differentiated cells (lane 3). In lane 2 is visualised the run of an aliquot of total cell lysate from differentiated cells. The migration of standard proteins is indicated.

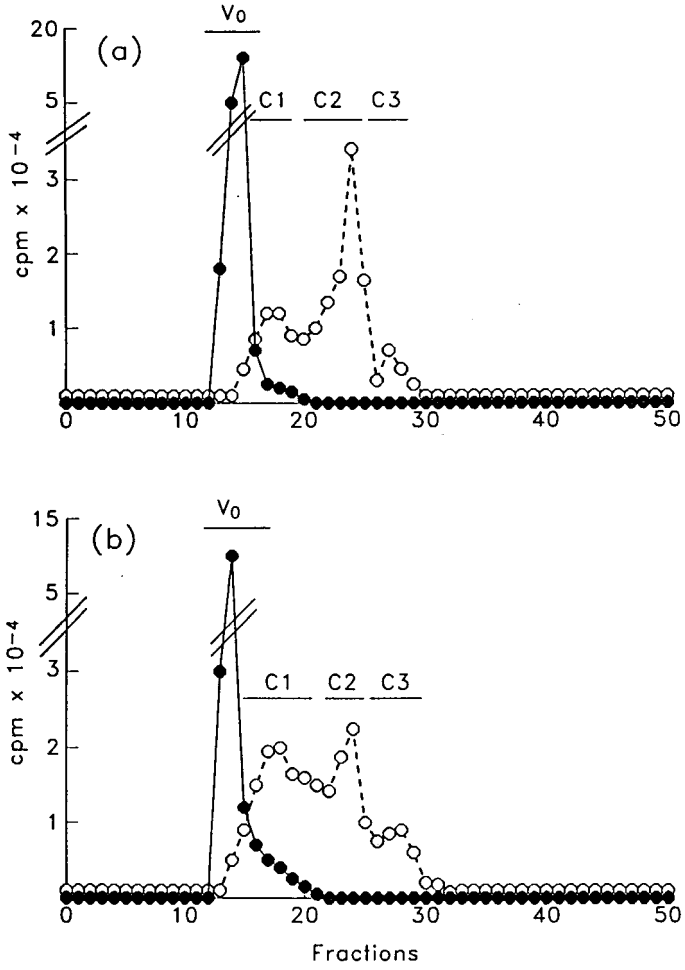

Figure 2. Bio-Gel P-4 filtration of Pronase-glycopeptides from [^{3}H]glucosamine-labelled gp190 synthesised by undifferentiated and differentiated Caco-2 cells, before and after mild alkaline borohydride treatment. Pronase glycopeptides were obtained as described in the text. (a) Profiles of Pronase-glycopeptides from gp190 synthesised by undifferentiated cells (●) and of glycans released by mild alkaline borohydride treatment from the corresponding glycopeptide fractions of V_0 (○); (b) Profiles of Pronase glycopeptides from gp190 synthesised by differentiated cells (●) and of glycans released by mild alkaline borohydride treatment from the corresponding glycopeptide fractions of V_0 (○). Glycans were pooled and designated as indicated by the horizontal bars.

into both gp190s was recovered into glycopeptides excluded from the Bio-Gel P-4 column (Fig. 2).

When [^{3}H]glucosamine-labelled glycopeptides from both gp190s were subjected to mild alkaline borohydride treatment, practically all the [^{3}H]glucosamine-derived radioactivity was recovered in three glycans species emerging in included volumes of the column (Fig. 2a and b). The released glycans were designated C1 (larger size), C2 (intermediate size) and C3 (smaller size). In terms of radioactivity, C1 glycans were more abundant in gp190 from differentiated cells than in that from undifferentiated cells (54% versus 34%) whereas C2 glycans predominates in gp190 from undifferentiated cells.

Degree of sialylation of C1-, C2-, and C3-glycans in gp190 from undifferentiated and differentiated Caco-2 cells

The *O*-glycans fractionated as in Figure 2a and b were separately subjected to DEAE-Sephacel chromatography in order to establish the degree of sialylation. Table 2 shows the relative distribution of neutral, mono- and disialylated forms in C1-, C2-, and C3-glycans from the two [^{3}H]glucosamine-labelled gp190s. C1- and C2-glycans appeared heterogeneous in respect of the degree of sialylation, very likely because of a significant overlapping of peaks, whereas the C3-glycans from both gp190s contained practically only neutral chains. In terms of radioactivity the total percentage of sialylated-glycans versus neutral-glycans was higher in gp190 from undifferentiated cells than in gp190 from differentiated cells.

Radioactive amino-sugar composition of C1-, C2- and C3-glycans in gp190 from undifferentiated and differentiated Caco-2 cells

As expected under mild alkaline borohydride treatment, the vast majority of terminal-reducing GalNAc of *O*-glycans was reduced to the corresponding alditol, that after acid hydrolysis was actually identified as GalN-OH and GlcNAc as GlcN. As Table 3 shows the ratio between [^{3}H]GlcN/[^{3}H]GalN-OH in C1-glycans of gp190 from differentiated and undifferentiated cells was 2.7 and 2.2, respectively, whereas a ratio near 1 was found in C2- and C3-glycans from both glycoproteins. Since each GalN-OH residue corresponds to a single *O*-linked chain it may be inferred that the large size of C1 is due to the addition of more than 2 carbohydrate-sequence containing GlcNAc, such as *N*-acetyllactosamine, in gp190 from differentiated and undifferentiated cells. On the other hand, C2- and C3-glycans of both gp190s should contain only a single *N*-acetyllactosamine unit. Taking into account that C1-glycans predominated in gp190 from differentiated cells, one may suggest that in these cells *N*-acetyllactosamine units preferentially elongate the glycoprotein.

Characterisation of *O*-glycans

A large body of evidence indicates that mucin-like proteins from cell membranes carry branched *O*-glycans, which diverge according to the sugar added to the peptide-bound GalNAc [27]. Among the various branching types that referred to as core-2, consisting in the addition of GlcNAc in β1,6-linkage to GalNAc of the Galβ1,3GalNAc innermost unit, has been extensively studied in different cell types and has been found to be actively stimulated upon differentiation process [28]. We assumed that C3-glycan was the precursor of all glycans carried by gp190. When the neutral C3-glycan from both gp190s was analysed by HPLC, it was almost entirely recovered at the retention time of the Galβ1,4GlcNAcβ1,6-(Galβ1,3)GalNAcOH tetrasaccharide (Fig. 3).

The predominant glycan carried by gp190 from undifferentiated cells is the monosialylated C2-chain (see Table 2). We

Table 2. Relative distribution of [^{3}H]glucosamine-labelled neutral, monosialylated and disialylated chains in C1-, C2- and C3-glycans from gp190 synthesised by undifferentiated and differentiated Caco-2 cells. C1-, C2-, and C3-O-glycans, fractionated as shown in Figure 2 were separately chromatographed on a DEAE-Sephacel column and eluted with a gradient of pyridine/acetic acid [19]. The percentage of neutral- mono- and disialylated chains of each chromatography was calculated on the basis of the radioactivity eluted as neutral, mono and disialylated glycan-markers and assuming as 100% the total radioactivity eluted in the corresponding chromatography. The values are means of two independent preparations of [^{3}H]glucosamine-labelled gp190s.

	% of radioactivity in gp190 glycans							
	From undifferentiated cells				*From differentiated cells*			
	C1	*C2*	*C3*	*Total*	*C1*	*C2*	*C3*	*Total*
Neutral chains	9	15	7	31	25	15	12	52
Monosialylated chains	12	30	1	43	18	15	1	34
Disialylated chains	14	12	0	26	11	3	0	14

Table 3. Ratio between [^{3}H]GlcN and [^{3}H]GalN-OH in C1-, C2- and C3-glycans of gp190 from undifferentiated and differentiated Caco-2 cells. C1-, C2- and C3-glycans were isolated as described in the legend of Figure 2 and the radioactive aminosugar composition was performed as previously described [18]. The values are means of two independent preparations of glycans from [^{3}H]glucosamine-labelled gp190s.

	[^{3}H]GlcN/[^{3}H]GalN-OH		
	C1	*C2*	*C3*
gp190 from undifferentiated cells	2.2	1.0	1.0
gp190 from differentiated cells	2.7	1.1	0.9

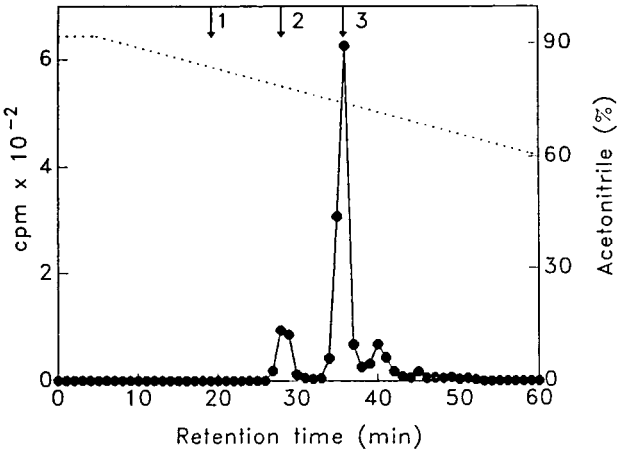

Figure 3. HPLC analysis of neutral C3-glycans from gp190. Neutral C3-glycan fractionated by DEAE-Sephacel chromatography was subjected to HPLC analysis as described in the text (system B). Arrows 1, 2 and 3 indicate the retention time of Galβ1,4GlcNAc, GalNAcβ1,4Galβ1,4GlcNAc, and Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcOH, respectively.

postulated that it represents the monosialylated homologous of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcOH. To prove this, the monosialylated C2-chain was subjected to neuraminidase digestion and then analysed by HPLC. In a preliminary experiment we had found that the vast majority of sialic acid bound to gp190s was susceptible to neuraminidase from NDV, an enzyme known to cleave specifically α2,3-linked sialic acid. After digestion with this enzyme the single peak of monosialylated C2-glycan was split in two peaks, one emerging at the retention time of *N*-acetylneuraminic acid (29% in term of radioactivity) and the other one at the elution time of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcOH (62% in term of radioactivity) (Figure 4). The relative distribution of radioactivity in the two components was consistent with the assumption that this glycan was the monosialylated homologous of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcOH. A very

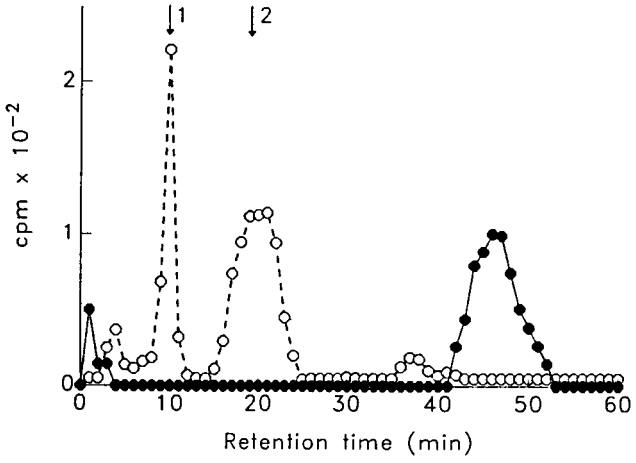

Figure 4. HPLC profiles of monosialylated C2-glycan after treatment with NDV-neuraminidase. HPLC profiles of monosialylated C2-glycan fractionated by DEAE-Sephacel chromatography before (●) and after NDV neuraminidase treatment (○). HPLC was performed as described in the text (system A). Arrows 1 and 2 indicate the retention time of *N*-acetylneuraminic acid and Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcOH.

similar result was obtained when the monosialylated C2-glycan from gp190 synthesised by differentiated cells was analysed (results not shown).

Finally, to prove that C1-glycans were elongated by *N*-acetyllactosamine units, the neutral C1-chains were digested with endo-β-galactosidase, an enzyme, which cleaves the *N*-acetyllactosamine unit, bound to the galactose residue. Figure 5 shows that the digestion resulted in the release of smaller components emerging from the Bio-Gel P-4 column in the elution volume of Galβ1,4GlcNAcβ1,6(Galβ1,3)-GalNAc-OH and of *N*-acetyllactosamine. In fact, neutral C1-chains were only partially cleaved, probably because the extensive substitution with fucose hindered the endo-β-galactosidase action. Even the neutral C1-chain of gp190 from undifferentiated cells was similarly susceptible to endo-β-galactosidase.

Glycosyltransferase activities

Current results indicate that gp190 *O*-glycans have the core 2-branched structure and are predominantly α2,3 sialylated. For this, the activities of core-2 β1,6GlcNAc-T, α2,3ST(*O*) and α2,3ST(*N*) responsible for the capping of the Galβ1,3GalNAc innermost-sequence and of the Galβ1,4GlcNAc elongating-unit, respectively, were investigated. As Table 4 shows the core-2 β1,6GlcNAc-T activity significantly increased upon Caco-2 cell differentiation, whereas no differentiation-dependent variance on the activity of two sialyltransferases was observed.

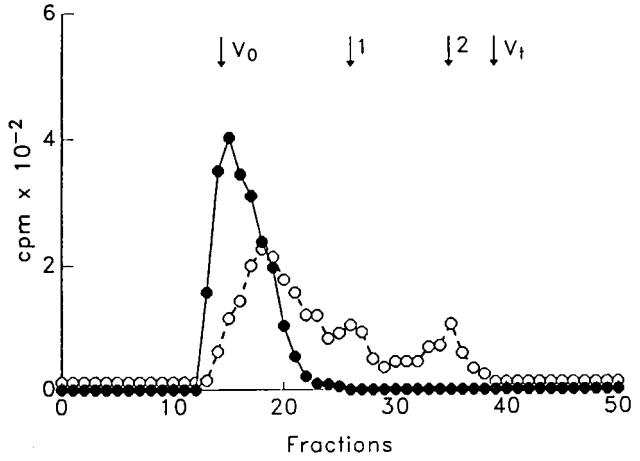

Figure 5. Bio-Gel P-4 filtration of neutral C1-glycan after treatment with endo-β-galactosidase. Gel-filtration profiles of neutral C1-glycan fractionated by DEAE-Sephacel chromatography untreated (●) and treated with endo-β-galactosidase (○). Arrows 1 and 2 indicate the elution position of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcOH and Galβ1,4GlcNAc, respectively.

Discussion

For the first time the characterisation of the glycomoiety of gp190 is reported. The major results are as follows: (i) gp190 carries for the vast majority *O*-glycans with the core-2 structure; (ii) a monosialylated homologous of the Galβ1,4GlcNAc-β1,6(Galβ1,3)GalNAc tetrasaccharide predominates in the glycoprotein synthesised by undifferentiated Caco-2 cells, whereas the tetrasaccharide is preferentially elongated by *N*-acetyllactosamine units in gp190 synthesised by differentiated cells. The larger elongation of glycans in gp190 from differentiated cells may explain the increment in the apparent molecular weight relative to that of gp190 from undifferentiated cells.

Many laboratories have analysed the glycosylation process of glycoproteins from undifferentiated and differentiated Caco-2 cells and significant alterations have been observed both in the activity of several glycosyltransferases and in the glycan assembly of individual glycoproteins [10–17]. Brockhausen *et al.* [13] have found a general increase in *O*- and *N*-linked branching glycosyltransferase activities upon Caco-2 cell differentiation, namely that of β1,6GlcNAc-transferase responsible for the assembly of core-2 *O*-glycans. We could confirm both the presence of the core-2 β1,6GlcNAc-transferase activity in undifferentiated cells and an increase in Caco-2 cell line maintained in culture for three weeks after the confluence (Table 4). Both results are consistent with the assembly of the Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAc tetrasaccharide in the two gp190s. On the other hand, since the enzymes responsible for the elongation by *N*-acetyllactosamine units were found to be unchanged upon the Caco-2 differentiation process [13], the predominance of polylactosaminoglycans in gp190 from differentiated cells cannot be merely related to the activation of these glycosyltransferases. It is possible that a higher assembly of the core-2 sequence in gp190 from differentiated cells may result in a better accessibility of the branched chain to the glycosyltransferases involved in the elongation by *N*-acetyllactosamine units.

Table 4. Glycosyltransferase activities in undifferentiated and differentiated Caco-2 cells. The activities were assayed as described in Materials and Methods. Means of two separate experiments.

		Glycosyltransferase activity (nmol/mg protein/h)	
Enzyme	*Acceptor*	*Undifferentiated cells*	*Differentiated cells*
α2,3-ST(O)	Galβ1,3GalNAc-*O*-Bn	0.32	0.29
α2,3-ST(N)	Galβ1,4GlcNAc	0.26	0.24
β1,6GlcNAc-T	Galβ1,3GalNAc-*O*-Bn	1.48	2.95

The glycan biosynthesis of lysosomal-membrane glycoproteins lamp-1 and 2 has been studied during the differentiation process of Caco-2 cells [12]. Only in the case of lamp-1 a decrease in fucosylated polylactosamino-glycans was observed during the differentiation process, strongly suggesting that the *O*-glycan processing follows different pathways in different glycoproteins. The fucosylation of gp190 has been previously demonstrated by the extensive labeling with [^{14}C]fucose [5]. In the present study such an aspect has not been evaluated nor its change under cell differentiation. Since in *O*-glycans the core-2 branching is critical for the biosynthesis of the sialyl-Lewis^a and sialyl-Lewis^x antigens [29], it is possible that the fucose residues and α2,3-linked sialic acid contribute to the expression of such antigens in gp190.

Our results indicate that gp190 from differentiated Caco-2 cells is less sialylated. The susceptibility of sialylated glycans from both gp190s to NDV neuraminidase strongly suggests that sialic acid is mainly present in α2,3-linkage. Current study confirms previous results indicating that the activity of the two α2,3-sialyltransferases involved in the capping of Galβ1,4GlcNAc or Galβ1,3GalNAc is not differentiation-dependent in Caco-2 cells [16]. It is worthy noting that even in colon carcinoma HT-29 cells no variation in the activity of α2,3-sialyltransferase responsible for the sialylation of glycosphingolipids has been found during the differentiation process [30]. Therefore, the differentiation-dependent sialylation of gp190 cannot be simply related to a change in the activity of sialyltransferases. Alternative mechanisms may explain the discrepancy between the glycosyltransferase expression and the glycosylation pathway of a protein. For instance, a difference in the rate of intracellular transit of a single glycoprotein resulting in a longer permanence in one of the compartment of the exocytic pathway may favour the action of a specific glycosyltransferase independently on the increment of the expression [31]. Wang *et al*. [32] have reported that in HL60 cells the assembly of polylactosaminoglycans attached to lamp-1 and lamp-2 is increased by the lowering the cell temperature, a condition that prolongs the permanence of glycoproteins into the Golgi apparatus. In Caco-2 cells a modified routing of gp190 may depend on the acquisition of the apical/basolateral polarity during the differentiation process. Hauri and his co-workers, in studies on the transport of intestinal microvillar hydrolases to the apical face of polarised Caco-2 cells, observed that the major slow down of routing could be ascribed to maturation in and transit of the enzymes through the Golgi apparatus and a market asynchronous transport was found of various enzyme [33,34]. In this contest, one may suggest that the transit of gp190 through the distal Golgi apparatus, where the sialyltransferases are localised, occurs in differentiated cells at a rate that interferes with the action of the sialyltransferases.

More recent observations [35,36] indicate that *O*-glycans are important for the sorting of integral-membrane or soluble glycoproteins to the apical face of polarised cells. It has been proposed that the *O*-glycans, namely those linked to the stalk region of the integral membrane glycoproteins could confer a conformation facilitating the integration of the glycoproteins to the apical membrane [35]. Indeed, steric interactions between the innermost GalNAc of *O*-glycans and the vicinal amino acids appears to modify the conformation of bovine and ovine mucins [37]. The role of the outer portion of *O*-glycans in modulating the fate of glycoproteins along the exocytic pathway has been poorly investigated, but recently a correlation between the α2,3-sialylation of mucins-like glycoproteins and their delivery to the apical surface in HT-29 colon carcinoma has been observed [38].

In conclusion the differentiation-dependent *O*-glycosylation of gp190 might be related to a different transit rate along the Golgi apparatus of cells which have acquired the apical/basolateral polarity, rather than to a modified expression of the Golgi glycosyltransferases responsible for the *O*-glycan elongation and sialylation. Further studies on the relationship between the carbohydrate assembly of gp190 and its transit rate into the Golgi compartments are required to substantiate this hypothesis.

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