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Relationship between the content of [^{14}C]glucose-derived monosaccharides in glycoprotein oligosaccharide chains and the state of enterocytic differentiation of HT-29 cells *

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ABSTRACT

The HT-29 cell line derived from a human colon adenocarcinoma has a glucose-dependent state of differentiation which is negatively correlated with the presence of D-glucose in the culture medium. The contribution of glucose to the biosynthesis of *N*-glycan chains, as a function of the differentiation state of HT-29 cells, was shown by: (a) [^{14}C]glucose incorporation by undifferentiated HT-29 cells being lower after 2 h and higher after 19 h of metabolic labeling than that by differentiated cells; (b) a lack of glucose in the culture medium of undifferentiated HT-29 cells diminishing [^{14}C]glucose incorporation into glycan chains, but not changing the glucose distribution between lipid- and protein-linked saccharides; (c) glucose behavior in undifferentiated HT-29 cells being not related to mannose-glycan metabolism, as the high-mannose compounds labeled with glucose and observed by HPLC showing a different distribution associated with the duration of glucose labeling; and (d) glucose being interconverted into other monosaccharide-glycan constituents in proportions different in differentiated and undifferentiated cell populations.

INTRODUCTION

Many in vivo and in vitro studies on tumor cells have focused attention on carbohydrate metabolism, which is associated with the neoplastic state[1], and glycoconjugate functions that actively influence fundamental biological processes, including cell proliferation, cell recognition, and cytodifferentiation[2]. It is generally accepted that glucose basically provides energy for the cell and is also an essential source of D-ribose 5-phosphate, a primary precursor in nucleic acid synthesis[3].

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Furthermore, sugars from the environment or neoglucogenesis and the hexamonophosphate cycle also participate in glycoconjugate-chain biosynthesis.

The HT-29 cell line was originally isolated by Fogh and Trempe[1,4] from a human adenocarcinoma of the colon. These cells, under various culture conditions, such as the presence of suramin[5], the addition of sodium butyrate[6], or the absence of glucose[7] replaced or not by galactose[8] or inosine[9], can differentiate into polarized monolayers of mucus-secreting or absorptive cells (or both), with an apical brush border membrane expressing hydrolase activities, for example, sucrase isomaltase, dipeptidyl peptidase IV, aminopeptidase N, and alkaline phosphatase.

The glucose-dependent state of HT-29 cell differentiation could be a consequence of cancer-associated deregulation of glucose uptake and metabolism. Gauthier et al.[10] reported that the differentiation of HT-29 cells is negatively correlated with the presence of glucose in the medium, as well as the degree of glucose consumption and lactic acid production. Moreover, we have previously shown[11] that the incorporation of [^{3}H]mannose into *N*-glycans was impaired in HT-29 cells cultured with inosine and glucose (undifferentiated conditions), as compared to that observed in differentiated HT-29 cells. Although both cell populations accumulated glycogen[9], undifferentiated HT-29 cells produced more aspartate and pyruvate. Regardless of the mechanism(s), these changes in intracellular glucose metabolism could play a role in *N*-glycan metabolism, but this remains to be demonstrated.

The aim of the present study was to assess the contribution of glucose, by its interconversion into other monosaccharides, to the biosynthesis of *N*-glycan chains as a function of the state of enterocytic differentiation of HT-29 cells.

EXPERIMENTAL

Cell culture.—The HT-29 cell line was established in permanent culture from a human colon adenocarcinoma by Fogh and Trempe[1,4]. The cells were routinely seeded at 1×10^5 cells/mL in plastic flasks (Falcon) with glucose-free Dulbecco's modified essential medium (DMEM), supplemented with 10% dialyzed, fetal-calf serum and 2.8 mM inosine (differentiated)[9]; further addition of 25 mM glucose maintained HT-29 cells in their undifferentiated state. Cultures were incubated at 37° in a humidified 5% CO_2 atmosphere. The medium was changed daily during the confluent and stationary (post-confluent) phases. The HT-29 cells cultured with inosine and without glucose were differentiated after reaching confluence (15 days)[12].

Cell labeling.—Cells were metabolically labeled with D-[U-^{14}C]glucose (286 mCi/mmol), D-[2-^{3}H]mannose (13.6 Ci/mmol) (The Radiochemical Centre Amersham, Bucks, UK). Inosine-differentiated HT-29 cells were labeled with 8 μCi/mL [^{14}C]glucose and inosine-glucose-undifferentiated HT-29 cells 10-fold more. The cells were metabolically labeled with [^{14}C]glucose for 2, 7.5, 9, and 19 h in the appropriate culture medium. HT-29 cells were labeled with D-[^{3}H]mannose for 6

h, with 40 μCi/mL for differentiated cells and 10-fold more for undifferentiated cells[11]. In order to determine whether glucose in the culture medium had an isotope dilution effect on the incorporation of radioactivity, undifferentiated HT-29 cells were labeled with 8 μCi/mL [^{14}C]glucose for 2 h in glucose-free medium.

Preparation and fractionation of glycopeptides and oligosaccharides.—After radioactive labeling, the cells were rinsed three times with M phosphate-buffered saline solution pH 7.4, (PBS), containing calcium and magnesium. Cell homogenates were then extracted successively with 2:1 (v/v) $CHCl_3$–MeOH and 10:10:3 (v/v/v) $CHCl_3$–MeOH–water to separate the lipid-linked monosaccharides and oligosaccharides from the glycoprotein fraction.

Glycopeptides were obtained from the delipidated pellet by Pronase digestion (2 mg/mL: grade CB, Calbiochem, Paris, France) at 60° for 18 h under a toluene atmosphere. Afterwards, they were fractionated on a Bio-Gel P-6 column (1 × 130 cm, 200–400 mesh, Bio-Rad, Paris, France) in 0.1 M pyridine acetate buffer, pH 5, containing 5 mM NaN_3. The void volume and the included volume of the column were determined with bovine serum albumin (BSA) and mannose, respectively. Four fractions were recovered, which were lyophilized and treated with endo-*N*-acetyl-β-D-glucosaminidase H (endo H, 2.5 munits) from *Streptomyces griseus* (Miles, France) in 50 mM citrate buffer (100 μL), pH 5, under a toluene atmosphere[13]. After endo H treatment, all the fractions were again filtered through a Bio-Gel P-6 column under the conditions described above. In both cell populations, only one fraction was endo H-sensitive (Fraction IV). This fraction, which contained the high-mannose-type glycans, was recovered and the released oligosaccharides were resolved by HPLC in a Varian model 5000 liquid chromatograph equipped with a 5-μm Aminospherisorb column (Société Française de Chromatographie, Neuilly-Plaisance, France) as previously reported[12].

Glucose interconversion into endo H-resistant glycans.—The endo H-resistant glycopeptides from only glucose-labeled cells were pooled (Fractions I, II, and III) and then hydrolyzed with 19:1 (v/v) acetic acid–10 M H_2SO_4 for 4 h at 80°. Afterwards samples were desalted and subjected to paper chromatography (Schleicher and Schüll, 2043b) in 5:3:2 (v/v) butanol–pyridine–0.10 M HCl. The radioactivity was determined with an automatic TII linear analyzer (Bertholt, Germany) connected to an Apple IIc computer.

Determination of protein content.—The protein contents were measured before Pronase treatment according to the method of Lowry et al.[14].

RESULTS AND DISCUSSION

Comparison of [^{14}C]glucose incorporation into glycopeptides from differentiated and undifferentiated HT-29 cells.—Radiolabeled-glucose incorporation into glycopeptides was measured after Pronase treatment (Table I). The results showed that for undifferentiated HT-29 cells the incorporation of the radioisotope was

TABLE I

Total [^{14}C]glucose incorporation into glycopeptides from differentiated and undifferentiated HT-29 cells [a]

Labeling time (h)	[^{14}C]Glucose in glycopeptide fraction (dpm/mg of protein)	
	Undifferentiated HT-29 cells	Differentiated HT-29 cells
2	1850 ± 43	17035 ± 130
7.5	54578 ± 233	70322 ± 265
9	29518 ± 171	32406 ± 180
19	187771 ± 433	35916 ± 189

[a] Undifferentiated (routinely cultured with inosine and glucose) and differentiated HT-29 cells (routinely cultured with inosine alone) incorporated [^{14}C]glucose (expressed in dpm/mg of protein) into their glycopeptide fractions.

dependent upon the duration of glucose labeling. Between 2 and 9 h of glucose labeling time, this cell population showed lower or similar radioisotope incorporation per weight of protein values compared to those for differentiated cells; only after 19 h of [^{14}C]glucose incorporation did this level differ greatly in undifferentiated cells.

When the radioactivity incorporated into both lipid- and protein-linked glycans was taken as 100%, the radioactivity of the glycopeptide fraction was 71 and 79% for differentiated HT-29 and undifferentiated cells, respectively. When undifferentiated cells were metabolically labeled in glucose-free medium for 2 h (the standard culture conditions for differentiated HT-29 cells), the percentage of radioactivity incorporated into the glycopeptide fraction did not change significantly (71 vs 79%). Nevertheless, the quantitative incorporation of [^{14}C]glucose into the glycopeptide fraction obtained from undifferentiated cells labeled in glucose-free medium was markedly lower than that in medium containing the sugar: 687 vs. 1850 dpm/mg of protein, respectively (see Table I). This rate of [^{14}C]glucose incorporation into glycopeptides was quite different when compared to that of [^{3}H]mannose. After 6 h of labeling with D-[^{3}H]mannose, the radioactivity of the glycopeptide fraction was 340 dpm/mg of protein in differentiated HT-29 cells, which represented 93% of the mannose incorporated (100% set as described above). In undifferentiated HT-29 cells, 436 dpm/mg of protein were incorporated into the glycopeptide fraction, representing 60% of the mannose incorporated. In addition, it should be noted that the presence of glucose in the culture medium of undifferentiated HT-29 cells did not interfere with the incorporation of mannose into the glycopeptide fraction[10].

Comparison of [^{14}C]glucose distribution in glycopeptides from differentiated and undifferentiated HT-29 cells.—After [^{14}C]glucose labeling, regardless of the cell population considered, the radioactivity was distributed in both endo H-sensitive and endo H-resistant glycans, as had been shown with [^{3}H]mannose-labeled glycopeptides[11]. The ratio of ^{14}C radioactivity between the endo H-sensitive

TABLE II

Distribution (%) of incorporated [^{14}C]glucose between endo H-resistant (R) and -sensitive (S) glycans in undifferentiated and differentiated HT-29 cells

Labeling time (h)	Undifferentiated HT-29		Differentiated HT-29	
	Endo H-R	Endo H-S	Endo H-R	Endo H-S
2	18	82	74	26
7.5	5	94	79	21
9	16	84	84	16
19	59	41	81	19

high-mannose-type fraction and endo H-resistant glycans was calculated from the sum of the radioactivities recovered in the glycopeptide fractions after Bio-Gel P-6 chromatography, taken as 100%. Regardless of the duration of labeling, a high proportion of ^{14}C radioactivity was found in endo H-resistant glycans from differentiated HT-29 cells. In undifferentiated HT-29 cells, on the other hand, between 2 and 9 h, the high [^{14}C]glucose proportion was found in the endo H-sensitive glycans but, after 19 h of labeling, the recovered radioactivity was more equally distributed in the endo H-resistant and -sensitive fractions (Table II). After 6 h of [^{3}H]mannose labeling, endo H-resistant and -sensitive glycans exhibited a distribution pattern similar to that found, after 7.5 h of labeling, for ^{14}C radioactivity in differentiated and undifferentiated HT-29 cells, i.e., 91% endo H-sensitive glycans in undifferentiated and 81% endo H-resistant glycans in differentiated cells. These ratios found in each cell population did not change, regardless of the time considered, when these cells were labeled with [^{3}H]mannose. These results showed that the glucose behavior in undifferentiated HT-29 cells did not affect mannose glycan metabolism. After 19 h of glucose labeling, ~ 60% of the ^{14}C radioactivity was found in endo H-resistant glycans, suggesting that the monosaccharides formed from [^{14}C]glucose correspond mainly to those present in endo H-resistant glycans in undifferentiated HT-29 cells. In order to further elucidate the fate of [^{14}C]glucose, we investigated the monosaccharide composition of the glycans.

[^{14}C]Glucose distribution in the high mannose fraction and its interconversion into other monosaccharides in the endo H-resistant glycans.—After endo H treatment, the [^{14}C]glucose- and [^{3}H]mannose(control)-labeled samples were filtered through Bio-Gel P-6, in order to separate endo H-resistant and -sensitive glycans. The high-mannose fractions (endo H-sensitive) of differentiated and undifferentiated HT-29 cells labeled for 7.5 and 19 h with [^{14}C]glucose and 6 h with [^{3}H]mannose were analyzed by HPLC.

In differentiated HT-29 cells, ^{14}C-labeled oligosaccharides were recovered in the $Man_5GlcNAc$, $Man_6GlcNAc$, $Man_7GlcNAc$, $Man_8GlcNAc$, and $Man_9GlcNAc$ fractions (Fig.s 1A and B). The following typical pattern had previously been found with [^{3}H]mannose[11]; the elution of high-mannose chains was observed regardless of the labeling time, in differentiated HT-29 cells labeled with [^{14}C]glucose. In

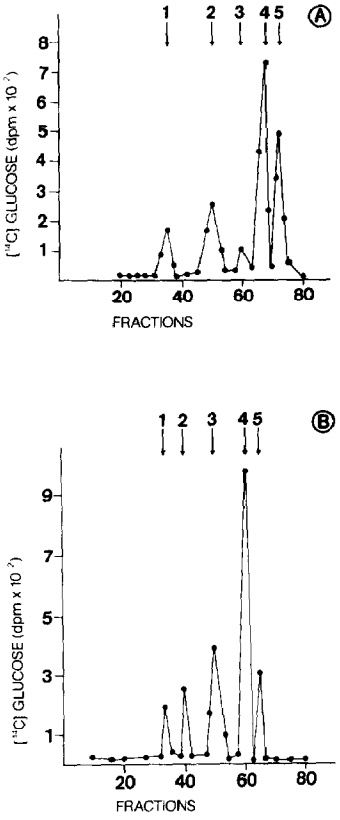

Fig. 1. HPLC of endo H-sensitive glycans from differentiated HT-29 cells labeled with [^{14}C]glucose. The arrows indicate the elution profile of standard ^{14}C-labeled oligosaccharides: (1) $Man_5GlcNAc$; (2) $Man_6GlcNAc$; (3) $Man_7GlcNAc$; (4) $Man_8GlcNAc$; and (5) $Man_9GlcNAc$. The oligosaccharides were labeled for 7.5 (A) or 19 h (B).

undifferentiated HT-29 cells, this pattern changed as a function of the labeling time; after 7.5 h of glucose labeling, only $Man_8GlcNAc$ and $Man_9GlcNAc$ were detected, whereas, after 19 h, all the oligosaccharides were labeled with [^{14}C]glucose (Figs. 2A and B). The controls performed with [^{3}H]mannose showed the typical pattern of oligosaccharide elution, regardless of the cell population considered (Figs. 3A, B).

The endo H-resistant glycans from both cell populations labeled with [^{14}C]glucose for 7.5 h were isolated and subjected to paper chromatography. Glucose was mainly converted into glucosamine and galactose (Table III), and the radioactivity was primarily recovered as glucosamine in undifferentiated and as galactose in differentiated cells. It had been previously demonstrated that [^{3}H]mannose is converted into fucose to the same extent in both cell populations[11], and that a high proportion of O-glycans is present in undifferentiated HT-29 cells[15]. The higher amount of radioactivity found in the *N*-acetylgalactosamine of undifferentiated

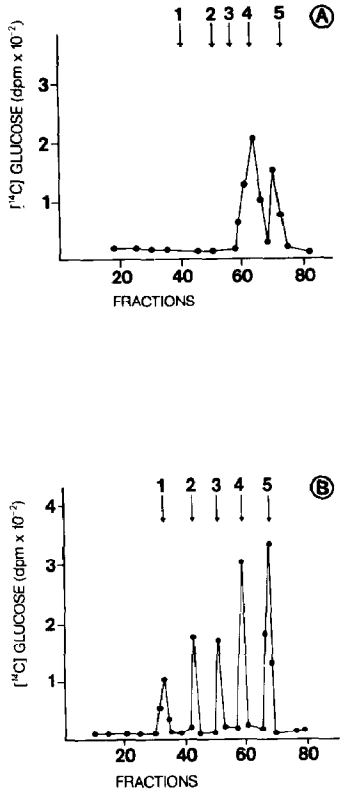

Fig. 2. HPLC of endo H-sensitive glycans from undifferentiated HT-29 cells labeled with [^{14}C]glucose. The arrows indicate the elution profile of standard oligosaccharides. For details, see the legend to Fig. 1. The oligosaccharides were labeled for 7.5 (A) or 19 h (B).

(9%) as compared to differentiated (5%) HT-29 cells confirmed that more O-glycans were present in the former cell population than in the latter.

The differences noted in the glycan distribution between differentiated and undifferentiated HT-29 cells were comparable to those observed in [^{3}H]mannose-labeled cells[12]. However, the distribution of [^{14}C]glucose after 19 h of labeling of undifferentiated HT-29 cells could be attributed to the characteristics of glucose metabolism or those of glycan metabolism (or both). It was previously established that undifferentiated HT-29 cells had a high rate of glycolysis and maintained high glycogen levels[16]. Despite this fact and regardless of the state of HT-29 cell differentiation, ^{14}C radioactivity was detected in oligosaccharide chains of glycoproteins. However, in undifferentiated cells, [^{14}C]glucose may preferentially enter the glycolytic pathway, as suggested by experiments with cells labeled in glucose-free medium. Furthermore, a higher variability of ^{14}C incorporated radioactivity ratio between endo H-resistant and -sensitive glycans was observed in undifferentiated cells when compared to differentiated cells. The relevance of these results to the metabolism of glycoproteins[17] and the high level of UDP-*N*-acetylhexosamine[9] observed in undifferentiated cells is of interest for further study.

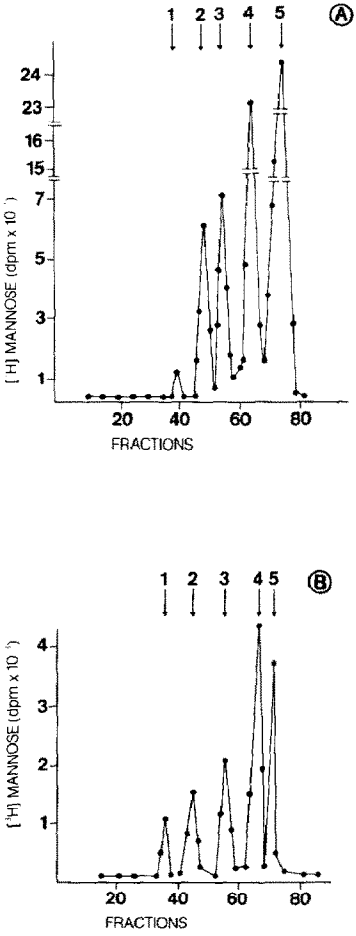

Fig. 3. HPLC of endo H-sensitive glycans from undifferentiated (A) and differentiated (B) HT-29 cells labeled with [^{3}H]mannose. The arrows indicate the standard oligosaccharides, for details see the legend to Fig. 1. The cells were radiolabeled with [^{3}H]mannose for 6 h and endo H-sensitive glycans were obtained as described in the Experimental section.

TABLE III

Radioactivity (%) in [^{14}C]glucose-derived sugars in endo H-resistant glycans [a]

Sugars	Undifferentiated HT-29 cells	Differentiated HT-29 cells
GalN	9	5
GlcN	10	9
Gal	7	14
Man	1	1

[a] The percentage of radioactivity of glucose-derived sugars were calculated as a function of the area found after linear counting of paper chromatograms, and mannose from [^{14}C]glucose incorporation was considered as 100%.

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REFERENCES

1 J. Fogh, J.M. Fogh, and T. Orfeo, *J. Natl. Cancer Inst.*, 59 (1977) 221–225.
2 H.J. Gabius, K. Vehemyer, R. Engelhardt, G.A. Nagel, and F. Cramer, *Cell Tissue Res.*, 246 (1986) 515–521.
3 B.M. Wice, L.J. Reitzer, and D. Kennel, *J. Biol. Chem.*, 256 (1981) 7812–7819.
4 J. Fogh and G. Trempe, in J. Fogh (Ed.), *Human Tumor Cells In Vitro*, Plenum Press, New York, 1975, pp 115–141.
5 J. Fantini, J.B. Rognoni, M. Roccabianca, G. Pommier, and J. Marvaldi, *J. Biol. Chem.*, 264 (1989) 10282–10286.
6 C. Augeron and C.L. Laboisse, *Cancer Res.*, 44 (1984) 3961–3969.
7 A. Zweibaum, M. Pinto, G. Chevalier, N. Triadou, B. Lacroix, K. Haffen, J.L. Brun, and M. Rousset, *J. Cell. Physiol.*, 122 (1985) 21–29.
8 M. Pinto, M.D. Appay, P. Simon-Assmann, G. Chevalier, G. Dracopoli, J. Fogh, and A. Zweibaum, *Biol. Cell.*, 44 (1982) 193–196.
9 B. Wice, G. Trugnan, M. Pinto, M. Rousset, G. Chevalier, E. Dussaulx, B. Lacroix, and A. Zweibaum, *J. Biol. Chem.*, 260 (1985) 139–146.
10 T. Gauthier, C. Denis-Pouxviel, and J.C. Murat, *Int. J. Cancer*, 21 (1989) 191–196.
11 E. Ogier-Denis, P. Codogno, I. Chantret, and G. Trugnan, *J. Biol. Chem.*, 263 (1988) 6031–6037.
12 E. Ogier-Denis, C. Bauvy, M. Aubery, P. Codogno, C. Sapin, M. Rousset, and A. Zweibaum, *J. Cell. Biochem.*, 41 (1989) 13–23.
13 P. Codogno, J. Botti, J. Font, and M. Aubery, *Eur. J. Biochem.*, 149 (1985) 453–460.
14 O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265–275.
15 E. Ogier-Denis, G. Trugnan, A.T. Tran, C. Sapin, M. Aubery, and P. Codogno, *C.R. Seances Hebd. Acad. Sci. Ser. III*, 310 (1990) 41–47.
16 T. Gauthier, C. Denis-Pouxviel, H. Paris, and J.C. Murat, *Biochim. Biophys. Acta*, 975 (1989) 231–238.
17 G. Trugnan, E. Ogier-Denis, C. Sapin, D. Darmoul, C. Bauvy, M. Aubery, and P. Codogno, *J. Biol. Chem.*, 266 (1991) 20840–20855.