CHANGES IN CELL SURFACE GLYCOPEPTIDES DURING MYOGENESIS IN WILD-TYPE AND NON-FUSING MUTANT L6 RAT MYOBLASTS

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1. Introduction

Established cell lines derived from skeletal muscle, exhibit many features of muscle differentiation in vitro. The mononuleated myoblasts divide actively. After reaching confluence, they stop synthesising DNA, align and fuse into multinucleated myotubes [1]. The process which leads to fusion includes at least two consecutive steps: specific cell–cell recognition; and membrane interactions which lead to the set up of intercellular connections and to a metabolic coupling between myogenic cells [2–5]. These studies and [6,7] indicate that specific changes take place on the cell surface of myogenic cells during this differentiation process [8–11].

Here we analysed the surface glycopeptides of myoblasts and myotubes of the L_6 line as well as those of a fusion-defective mutant of this line (Ama102). We show numerous differences in the size distribution and carbohydrate composition of the externally exposed glycopeptides obtained from myogenic cells at different stages of differentiation.

2. Materials and methods

2.1. *Cell cultures*

Culture of cells of the line L_6H_9 and of a non-fusion mutant of this line having an altered RNA polymerase II (Ama102) was done as in [12].

2.2. *Labelling of the cells and obtention of tryptic surface glycopeptides*

At various stages of myogenesis, the cells were labelled at 37°C in 10 ml of Dulbecco's modified Eagle medium/plate of diam 100 mm leucine, 10% foetal calf serum with 2.5 μCi/ml [1-^{14}C]glucosamine (spec. act. 57 mCi/mmol, CEA France) and 10 μCi/ml [^{3}H]leucine (spec. act. 35 Ci/mmol, CEA France). After 24 h, radioactive media were removed and the cultures were washed several times with Ca-Mg free Dulbecco and exposed to 0.25% trypsin in Ca-Mg free Dulbecco for 1 h at 37°C. Subsequently a 2-fold excess of purified soybean trypsin inhibitor (Sigma) was added and the medium was separated from the cells by centrifugation for 10 s at 2500 rev./min. The pellet was used to measure DNA content [13]. The supernatant of 20 cultures dishes (100 mm diam.), which contains the solubilized glycopeptides, was concentrated by ultrafiltration on a PM 0.5 Amicon filter and fractionated on a Sephadex G-25 column (2.5 × 50 cm) equilibrated in NH_4CO_3 1 mM. The excluded fraction was concentrated on a PM 0.5 Amicon filter and submitted to a second filtration on a Sephadex G-100 column equilibrated with the same buffer. Fractions of 5 ml were collected in both cases.

2.3. *Isolation of glucosamine, galactosamine and sialic acid*

The various monosaccharides were isolated and quantitated as in [14]. Unlabelled carbohydrates were added to the radiolabelled solutions and the radioactivity associated with each peak of cold carbohydrate was measured and expressed as a % of the total carbohydrate/fraction.

3. Results and discussion

As shown in fig.1, the heavily glycosylated surface peptides obtained from mononucleated L_6 myoblasts were excluded upon chromatography on a Sephadex

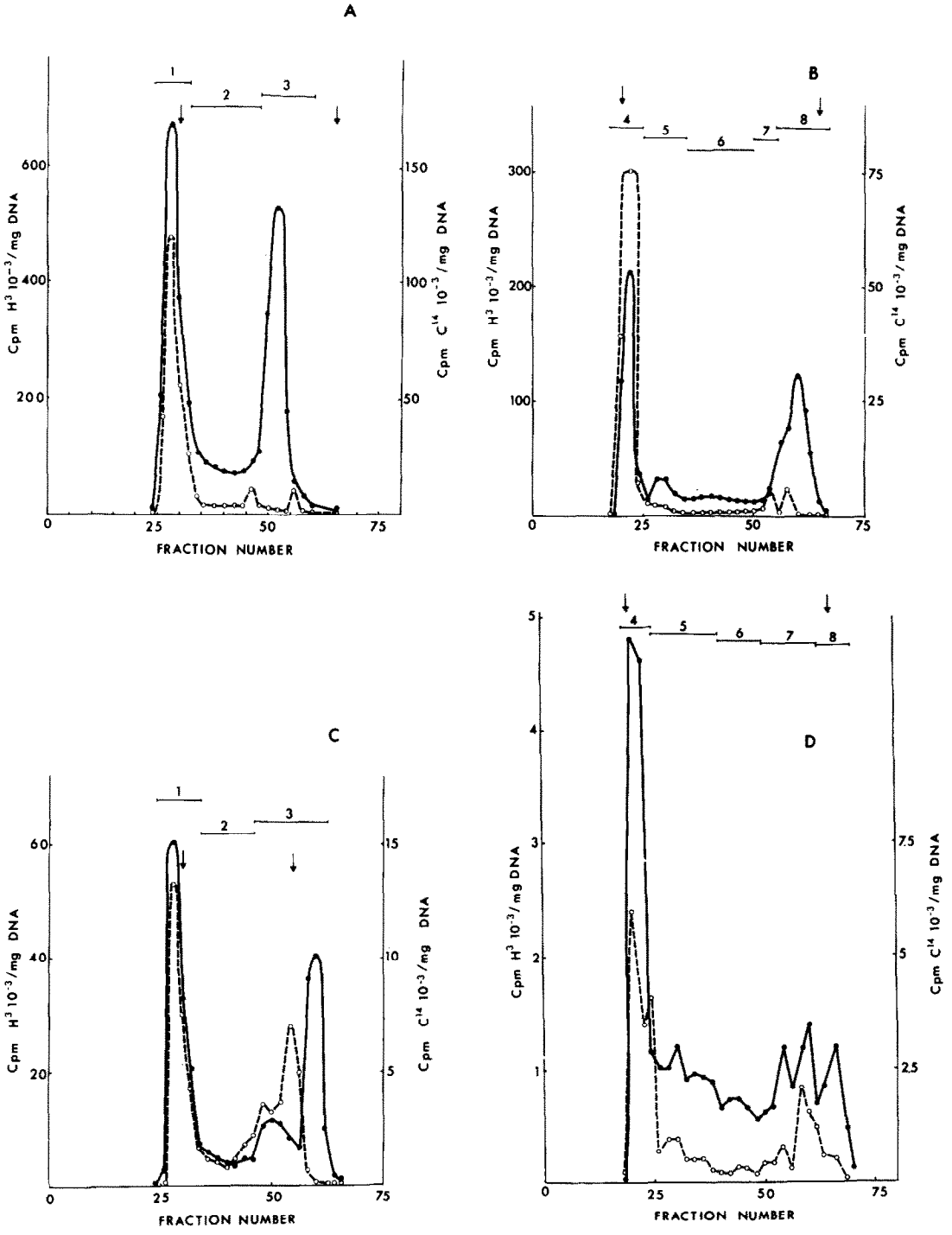

Fig.1. Gel filtration of surface glycopeptides of cells of the line L_6H_9. Cells of the line L_6H_9 were cultured and labelled with [^{3}H]-leucine (●——●) and [^{14}C]glucosamine (○--○) as in section 2. The surfac glycopeptides from myoblasts (6 X 10^5 cells/100 mm diam. plate) (A,B) and fused cultures (85% nuclei in myotubes) (C,D) were obtained as in section 2. They were filtred on a Sephadex G-25 column (A,C). Peak 1 was submitted to a Sephadex G-100 (B,D). The results are expressed per milligram of DNA.

Table 1
Carbohydrate content of various fractions of chromatography of line L_6H_9

Origin	Fraction		Glucos-amine (%)	Galactos-amine (%)	Sialic acid (%)
Myoblasts	A_2	LMW	49	51	n.d.
(6×10^5 cells/	A_3		n.d.	n.d.	n.d.
100 mm diam. plate)	B_4		41	49	10
	B_5	HMW	60	40	n.d.
	B_6 B_8		>90	n.d.	n.d.
Myotubes	C_2	LMW	40	51	9
(85% of nuclei	C_3		43	55	5
in myotubes)	D_4		51	42	7
	D_5	HMW	n.d.	n.d.	n.d.
	D_6		n.d.	n.d.	n.d.
	D_7		>90	n.d.	n.d.
	D_8		>90	n.d.	n.d.

G-25. This class of molecules also eluted in the excluded volume upon a second filtration on Sephadex G-100.

In contrast, a high proportion of the glycopeptides obtained from the surface of multinucleated myotubes were eluted in the included volume of a Sephadex G-25 column (fraction 3, fig.1C). Note that the net incorporation of both leucine and glucosamine was reduced roughly 5-fold in myotube vs myoblast cultures.

The incorporated glucosamine/leucine ratio of the high molecular weight peptides obtained from both types of cells was determined. It was of 1:5 for myoblasts and 1:1 for myotubes (fraction 4, fig.1B,D). As shown in table 1, the low molecular glycopeptides from myoblasts contain glucosamine and galactosamine in about the same proportions. However, in myotubes, the corresponding classes of glycopeptides contain in addition sialic acid. This carbohydrate is found in mononucleated L_6 cells only in the high molecular weight fraction.

As far as the mutant cells of line Ama102 are concerned, ~75% of the surface glycopeptides were eluted in the included volume of a Sephadex G-25. The ratio of incorporated glucosamine:leucine in these glycopeptides was about 1:5 (fig.2A,2C, fraction 3). The excluded fraction from the Sephadex G-25 column was further resolved on a Sephadex G-100 column. The elution patterns of the glycopeptides from non-confluent and confluent cells of this mutant line differed from fig.1; a marked decrease of the class of excluded peptides was observed at confluency. Carbohydrate analysis of the low molecular weight fractions showed that the ratio of glucosamine: galactosamine in fraction 2 was roughly 1:4, while it was ~1:1 in wild type cells. Fraction 3 obtained from non-confluent Ama102 cells was found to be extremely rich in sialic acid (83%), while the corresponding class of glycopeptides obtained from confluent cells exhibited a lower content in this carbohydrate (27%) (table 2). Interestingly enough, the corresponding class in L_6 cells was either practically not glycosylated (myoblasts), or contained an extremely low percentage of sialic acid (myotubes) (table 1). In the high molecular weight glycopeptides, relatively more sialic acid was found in confluent cells than in non-confluent ones (table 2).

Several conclusions can be drawn from the results presented above:

(1) The surface glycopeptides of multinucleated myotubes are more heavily glycosylated than those from mononucleated cells of the line L_6.
(2) Several new size classes of glycopeptides appear on the surface of these cells during myogenesis.
(3) Comparison with one non-fusion mutant shows that the surface of these latter cells exhibit a different size distribution of glycopeptides some of which, contain a large proportion of sialic acid.

The differences in carbohydrate composition and glycopeptide distribution could be responsible for the fusion of the cells of the line L_6 and the loss of this property in mutant cells of the line Ama102.

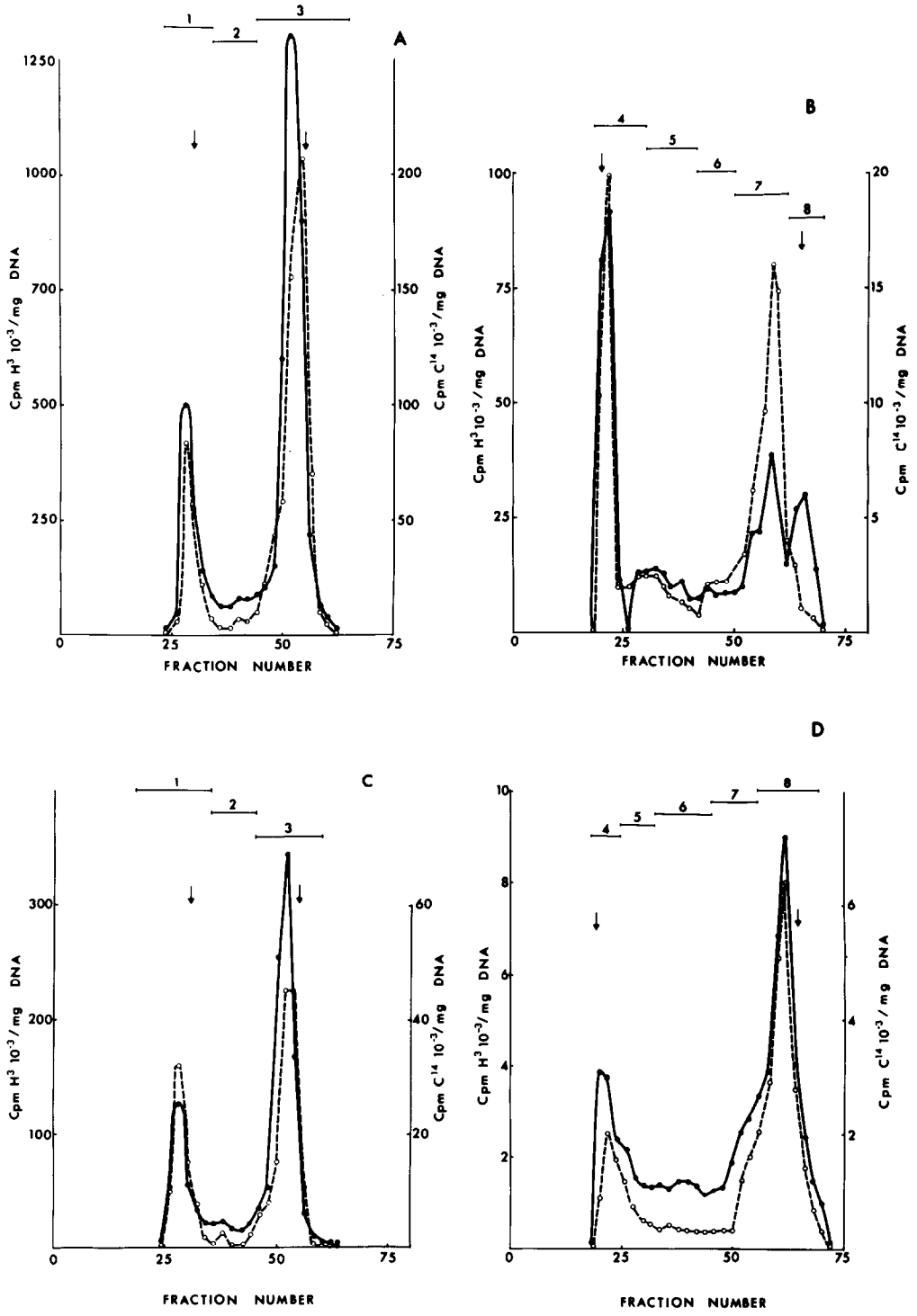

Fig.2. Gel filtration of surface glycopeptides of cells of the non-fusing line Ama102. Cells of the line Ama102 were cultured and labelled with [^{3}H]leucine (•——•) and [^{14}C]glucosamine (○--○) as in section 2. The surface glycopeptides from non-confluent cells (A,B) (6 × 10^5 cells/plate of 100 mm diam.) and confluent cells (C,D) (10^5–10^6 cells/plate of 100 mm diam.) were obtained as described (see section 2). They were filtered on a Sephadex G-25 column (A,C). Peak 1 was submitted to a Sephadex G-100 (B,D). The results are expressed per picogram of DNA.

Table 2
Carbohydrate content of various fractions of chromatography of Ama102 line

Origin	Fraction		Glucos-amine (%)	Galactos-amine (%)	Sialic acid (%)
Subconfluent cells	A_2	LMW	19	81	n.d.
(6×10^5 cells/	A_3		3	14	83
100 mm diam. plate)	B_4		66	33	n.d.
	B_5		46	54	n.d.
	B_6	HMW	n.d.	n.d.	n.d.
	B_7		>90	n.d.	n.d.
	B_8		n.d.	n.d.	n.d.
Confluent cells	C_2	LMW	17	83	n.d.
(1.5×10^6 cells/	C_3		26	47	27
100 mm diam. plate)	D_4		55	35	10
	D_5	HMW	n.d.	n.d.	n.d.
	D_6		n.d.	n.d.	n.d.
	D_7		81	19	n.d.
	D_8		78	22	n.d.

Abbreviations: LMW, low molecular weight fractions; HMW, high molecular weight fractions; n.d., not detectable (<5%)

The letters and numbers of both tables correspond to the various chromatographic fractions on fig.1,2

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