Glycoconjugate J (1986) 3: 217–219

Herpes Simplex Virus Type 1 Glycoprotein C Synthesized in Ricin-resistant Cells Lacking *N*-Acetylglucosaminyltransferase I Accumulates $Man_5GlcNAc_2$ Glycans. A Correction

F DALL'OLIO[1], N MALAGOLINI[1], G CAMPADELLI-FIUME[2] and F SERAFINI-CESSI[1]

[1]*Istituto di Patologia Generale, and* [2]*Istituto di Microbiolgia e Virologia, Università di Bologna, Via S. Giacomo 14, 40126 Bologna, Italy*

Received April 28, 1986.

In the previous issue of this Journal we reported [1] on the characterization of *N*- and *O*-linked glycans carried by herpes simplex virus type 1 (HSV-1) glycoprotein C (gC) synthesized in a clone of ricin-resistant cells (Ric^R14) defective in *N*-acetylglucosaminyltransferase I [2]. The gC from mutant cells was found to carry *N*-linked glycans of high-mannose type, whereas the extent of *O*-glycosylation did not appear to be different from that of the glycoprotein synthesized in wild type cells. The strong reduction in the release of progeny virus from infected Ric^R14 cells was related to the absence of complex type glycans in the viral glycoproteins. Specifically, in that study we reported that the major species of high mannose chains accumulating in gC from Ric^R14 cells was $Man_4GlcNAc_2$. This result was unexpected because the glycan most frequently found in glycoproteins produced by cells lacking *N*-acetylglucosaminyltransferase I is $Man_5GlcNAc_2$ [3-5]. Our assertion that gC from mutant cells accumulated $Man_4GlcNAc_2$ was based on consistent results obtained by TLC analysis. In the system we used [6], the major oligomannoside released by endo-β-*N*-acetylglucosaminidase H (endo-H) from [^{3}H]mannose-labeled glycoprotein migrated faster than an authentic $Man_5GlcNAc$ standard which had been labeled with tritiated borohydride.

While the paper [1] was in press, we analyzed the same oligomannoside sample by HPLC and observed that it was eluted from the column with the retention time of an unlabeled $Man_5GlcNAc$ unit (Fig. 1). The discrepancy between the two results suggested that the TLC analysis was inaccurate in that the mobility of the oligomannoside released by endo-H from gC was compared with that of oligomannoside standards which had been converted to the corresponding alditols by tritiated borohydride treatment.

Indeed, when we examined the TLC mobility of a preparation of $Man_{9\text{-}5}GlcNAc$ glycans before and after the reductive action of borohydride, we found that the reducing forms migrated faster than the corresponding alditols. This result clearly explains our

Abbreviations are as follows: M_5, $Man_5GlcNAc$; M_6, $Man_6GlcNAc$; M_7, $Man_7GlcNAc$; M_8, $Man_8GlcNAc$; M_9, $Man_9GlcNAc$.

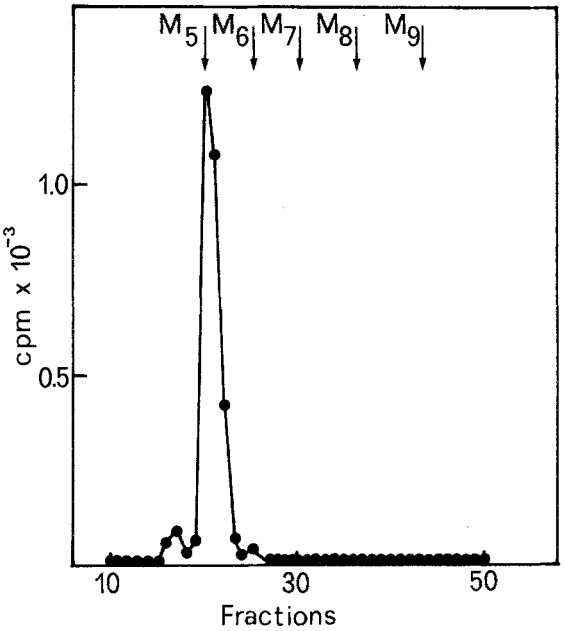

Figure 1. Separation by HPLC of [^{3}H]mannose-labeled oligosaccharides released by endo-H from gC synthesized in HSV-1-infected Ric^{R}14 cells.

HSV-1-infected Ric^{R}14 cells were continuously labeled with [^{3}H]mannose-labeled from 7 to 18 h after infection and chased for 3 h in the absence of the labeled mannose as described [1]. [^{3}H]mannose-labeled gC was purified by affinity chromatography on HC1 monoclonal antibodies linked to Sepharose and the Pronase-digested glycopeptides were treated with endo-H as described [1]. The released [^{3}H]mannose-labeled oligosaccharides were desalted by coupled columns of Dowex-50 and Dowex-1 and injected in a column (0.4 × 25 cm of Lichrosorb Diol 10 μm, Merck, Darmstadt, W. Germany) with unlabeled $Man_{9\text{-}5}GlcNAc$ standards prepared from unit A of thyroglobulin according to Godelaine *et al.* [6]. Acetonitrile/water, 70/30 by vol, was used as an eluent at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected and monitored for radioactivity to detect the [^{3}H]mannose-labeled components. The elution volumes of the unlabeled oligomannoside standards were detected by the absorption at 200 nm. About 90% of the radioactivity was recovered in the elution position of the $Man_5GlcNAc$ standard, whereas 5% of the radioactivity was eluted with a shorter retention time.

misinterpretation in assigning a $Man_4GlcNAc$ size to a glycan which is, in fact, $Man_5GlcNAc$.

Hughes and Mills [3] found that in uninfected Ric^{R}14 cells the biosynthesis of *N*-linked glycans is halted at the stage of $Man_5GlcNAc_2$. The present result confirms their evidence and clearly indicates that *N*-glycan biosynthesis of HSV-1 glycoproteins is strictly dependent on the host cell glycosyltransferases.

Acknowledgements

We thank M. Martelli for typing the manuscript. The excellent technical assistance of G. Bellabarba and A. Dalla Casa is also acknowledged. This work was supported by Consiglio Nazionale delle Ricerche, Progetto Finalizzato Oncologia, grant no. 85.02364.44, and Progetto Finalizzato Ingegneria Genetica, grant no. 85.01396.51.

References

1 Dall'Olio F, Malagolini N, Campadelli-Fiume G, Serafini-Cessi F (1986) Glycoconjugate J 3:71-84.
2 Vischer P, Hughes RC, (1981) Eur J Biochem 117:275-84.
3 Li E, Kornfeld S (1978) J Biol Chem 253:6426-31.
4 Hughes RC, Mills G (1983) Biochem J 211:575-87.
5 Hsieh P, Rich Rosner M, Robbins PW (1983) J Biol Chem 258:2555-61.
6 Godelaine D, Spiro MJ, Spiro RG (1981) J Biol Chem 256:10161-68.