Appearance of smaller mannosyl-glycopeptides on the surface of a human cell transformed by simian virus 40

(surface glycopeptides/growth-dependent alterations/cell transformation)

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ABSTRACT When fucosyl surface glycopeptides from growing normal human cells (WI 38) were compared with those derived from nongrowing cells the former were enriched in high-molecular-weight species. However, a line of human cells (WI 18Va) transformed by simian virus 40 appeared to have fucosyl-glycopeptides similar in size distribution to those from rapidly growing non-transformed cells (WI 38). I propose that the enrichment in high molecular weight species in these cells might be growth- rather than transformation-dependent. Using radioactive mannose to label surface glycopeptides, I observed that those derived from transformed cells (WI 18Va) were smaller than those from rapidly growing normal cells. Thus differences in size distribution may not be adequate criteria to evaluate the growth-dependent alterations in cell surface glycopeptides.

Normal cells in culture, unlike cancer cells or virus transformants, show "contact inhibition" of growth, i.e., the population density stabilizes at relatively low levels (1–5), the precise value varying with the individual cell line, pH (4), growth conditions (5), and serum concentration (6). Regulation of cell growth is thought to involve alterations of the cell surface (7). The changes on the cell surface can be monitored indirectly by the use of plant lectins or directly by analyzing the glycolipids and/or glycoproteins (see Tooze, ref. 8, for a review). To study surface glycoproteins cells are usually labeled with carbohydrate precursors such as glucosamine and fucose, followed by either the isolation of the plasma membrane or removal of surface glycopeptides by mild proteolytic digestion (9, 10). After extensive digestion with Pronase the analyses of the glycopeptides are carried out by gel filtration chromatography to examine the molecular weight distribution. All studies have shown an enrichment in high-molecular-weight glycopeptides in virus-transformed compared to nontransformed cells (9–15) and also in growing compared to non-growing cells (11, 14, 16).

I have compared the size distribution of surface glycopeptides derived from a normal human fibroblast (WI 38) and a cell line (WI 18Va) transformed by simian virus 40 (SV40). The data show that various labeled carbohydrate precursors must be used to fully evaluate the surface glycopeptides with respect to size alteration in SV40-transformed cells, since transformation induces smaller mannosyl surface glycopeptides.

MATERIALS AND METHODS

WI 38 and WI 18Va were obtained from Drs. Strecker and Eagle (Albert Einstein College of Medicine), respectively. The cells were grown in Eagle’s minimum essential medium (17) without antibiotics, supplemented with 5% calf and 5% fetal-calf serum (Grand Island Biological Co., Grand Island, N.Y.). Stock cultures were fed every other day and on the day before subculture. The cells were tested for mycoplasmal infections (18) and found to be negative. Experimental cultures were plated at a density of 1.0 to 1.5 X 10⁵ cells per cm² in T15 closed culture flasks (19) and after 24 hr fed daily with medium buffered at pH 7.6 WI 38 and at pH 7.4 for WI 18Va (20). pH varied by only ±0.2 pH units during periods between feeding.

The cells were labeled with L-[³H]fucose (5 μCi/ml specific activity 13.4 Ci/mmol), D-[³H]glu- cose (5 μCi/ml specific activity 2 Ci/mmol), L-[¹⁴C]fucose (0.2–0.5 μCi/ml specific activity 56.2 Ci/mole), and D-[¹⁴C]mannose (0.2–0.5 μCi/ml, specific activity 240 Ci/mole). The radioactive fucose was purchased from New England Nuclear, the [¹⁴C]mannose from Schwarz-BioResearch Inc. and the [³H]mannose from Amersham Radiochemical Centre. At the end of the labeling period (20–24 hr) the surface glycopeptides were obtained from the cells by mild protease release as previously described (16) by treatment with Pronase (100 μg/ml at room temperature) for 7 min. I consistently observed that these two cells (WI 38 and WI 18Va) adhered more tightly to the glass than KL2, another human diploid cell (16). The released glycopeptides (surface material) were further digested for a total of 48 hr at 37°C with Pronase (5 mg/ml) to ensure exhaustive removal of amino acids and prepared for Sephadex G-50 (0.9 cm X 120 cm) chromatography as previously described (16). Elution was carried out with 0.04 M ammonium acetate, pH 6.0. 0.5 ml fractions were collected and analyzed in 2.5 ml of Aquasol (New England Nuclear) with a Packard scintillation spectrometer. No radioactivity was excluded and only two included peaks of radioactivity were detected, one around 3000 in molecular weight and the other free monosaccharides.

RESULTS

WI 38, a human fibroblast, grows optimally at pH 7.6 (21) and under these conditions with daily feeding reaches a final density (growth inhibition) of approximately 4 X 10⁵ cells per cm² after 12–14 days. The cells were labeled with radioactive carbohydrate precursors during the log phase (2–3 days after plating) and during the stationary phase (13–14 days after plating). WI 18Va is a human SV40 transformant cell line. It grows to much higher cell densities than the normal fibroblast and, although the cells appear to develop a growth inhibitory effect, they never reach a truly stationary phase because, in contrast to the untransformed cells, they consistently detach from the glass as a coherent sheet, 8–10
days after reaching confluency. This phenomenon is characteristic of many cell lines (22). Therefore, I have labeled WI 18Va only during the log phase, that is, when approximately 75% of the surface area of the T15 flask was covered by cells.

Fucose did not label other hexoses in 20–24 hr, as determined by hydrolysis of glycopeptides and paper chromatography (16). However, after the same labeling period, about 14% of the radioactive mannose was found to comigrate with authentic fucose; the remaining 86% chromatographed with authentic mannose.

**Fucose-Labeled Surface Glycopeptides.** Cell surface material from growing WI 38 cells (labeled with [3H]fucose) was mixed with that from nongrowing cells (labeled with [14C]fucose) and after extensive digestion with Pronase the glycopeptides were chromatographed on a Sephadex G-50 column. The radioactive glycopeptides eluted between fractions 85 and 125 (Fig. 1). As noted earlier in several laboratories, glycopeptides from the surface of growing cells were enriched with higher molecular weight species (about 5500 molecular weight) when compared to those prepared from nongrowing cells (Fig. 1A). However, when surface glycopeptides from growing WI 38 cells (labeled with [3H]fucose) were compared with those removed from the surface of WI 18Va (labeled with [14C]fucose) similar chromatographic profiles were obtained (Fig. 1B). Thus, the surface glycopeptides derived from rapidly growing normal human diploid cells have a size distribution similar to those removed from SV40-transformed cells, and I conclude that in these cells fucosyl-glycopeptide size differences seem growth-dependent rather than transformation-dependent.

**Mannose-Labeled Surface Glycopeptides.** Pronase-digested surface glycopeptides from log phase WI 38 cells (labeled with [14C]mannose) and those from nongrowing cells (labeled with [3H]mannose) were also compared by Sephadex G-50 chromatography (Fig. 2A). The glycopeptides from the growing cells had a broader profile than those from nongrowing cells. It appears that the mannosyl-glycopeptides from the log phase cells were more heterogeneous than fucosyl-glycopeptides, in both larger (shoulder at about fraction 100) and smaller (fractions from 120 to 130) species than those derived from nongrowing cells (Fig. 2A).

Mannosyl-glycopeptides from the surface of the SV40-transformed cells (WI 18Va) were strikingly smaller in the average size distribution of the glycopeptides than those derived from the surface of growing WI 38 cells (Fig. 2B). It is noted that when fucose was used as a label no size difference was observed between the surface glycopeptides from grow-
ing WI 38 cells and those from WI 18Va cells (Fig. 1B). I conclude that certain species of mannosyl-glycopeptides are reduced in size in SV40-transformed cells.

DISCUSSION

Several laboratories (9-16) have demonstrated that in virus-transformed and rapidly growing cells there is an enrichment in higher molecular weight surface glycopeptides in comparison to nontransformed and contact-inhibited cells (nongrowing), respectively. All of these laboratories have used radioactive fucose and glucosamine to label the surface of cells. Using radioactive fucose I have confirmed only that such enrichments appear growth-dependent. The enrichment does not appear to be transformation-dependent, i.e., the surface glycopeptides from rapidly growing normal cells have the same size distribution as those derived from transformed cells. Two explanations are possible: (i) transformation of human cells by SV40 causes surface alterations different from those occurring with mouse, hamster, and chick cells. (ii) The cells in these studies were labeled for only 20-24 hr during early log phase (16) and not for 2-5 days during growth (9-15). In many “contact-inhibited” cells, in contrast to transformed or cancer cells, the rate of growth slows down considerably 3-4 days after plating (22). Thus the preparation of surface glycopeptides, after a long labeling period, might represent a population derived from rapid and slow growing cells.

When radioactive mannose was used, although rapidly growing and nongrowing WI 38 cells behaved in the expected manner, the virus-transformed cells contained mannosyl surface glycopeptides which were smaller than in rapidly growing normal cells (Fig. 2B). Thus, surface alterations in transformed cells do not always lead to larger glycopeptides and I suggest that the differences in size distribution must be carefully defined in evaluation of growth-dependent alterations in cell surface glycopeptides. We have evidence, from another normal human diploid fibroblast, that the mannosyl surface glycopeptides acquire an altered core oligosaccharide structure as the cells go from the growing to the nongrowing state, possibly due to altered ratios of glycopeptides of thyroglobulin unit A and unit B types (23). However, growth-dependent and transformation-dependent alterations in the oligosaccharide moiety of cell surface glycopeptides are complex phenomena and are not readily described by molecules’ simply being larger or smaller. The relationship of such alterations to the primary events in growth regulation remains obscure.

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