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Comparison of N-Acetylglucosaminyltransferase V Activities in Rous Sarcoma-Transformed Baby Hamster Kidney (RS-BHK) and BHK Cells

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Recent studies have desmonstrated that Rous sarcoma virus-transformed baby hamster kidney (RS-BHK) cells express twofold higher levels of those N-linked oligosaccharides that contain the sequence [GlcNAc-β(1,6)Man (1,6)] compared to nontransformed parental BHK cells (Pierce and Arango, *J. Biol.Chem. 261*, 10772 [1986]). We have investigated in RS-BHK and BHK cells the activity of UDP-GlcNAc:α-D-mannoside β(1,6)N-acetylglucosaminyltransferase V, the enzyme that begins the synthesis of the sequence that is increased in the RS-BHK cells.We have measured GnT V activity using UDP-[^{3}H]-GlcNAc and a synthetic oligosaccharide acceptor, GlcNAcβ(1,2)Man α(1,6)Manβ-O-$(Ch_2)_8COOCH_3$, separating the radioactive product by a newly devised reverse-phase chromatographic technique. Assayed under optimal conditions, the specific activity of GnT V is about fourfold higher in RS-BHK sonicates than in BHK sonicates, suggesting that this increase in activity may be the primary mechanism that causes the increase in [GlcNAcβ](1,6)Man] sequences in the RS-BHK cells. The apparent K_m values of the enzymes in RS-BHK and BHK cell sonicates for UDP-GlcNAc and the synthetic acceptor are similar, as are the pH optima. These results suggest that the increase in GnT V-specific activity in RS-BHK cells is not caused by the presence in these cells of a GnT V with markedly different kinetic properties.

Key words: glycosyltransferase, cell surface, transformation

Baby hamster kidney (BHK) fibroblasts transformed independently by two dissimilar tumor viruses, Rous sarcoma and polyoma, show a similar significant alteration in the expression of a particular subclass of N-linked oligosaccharides [1,2]. Both Rous sarcoma-transformed (RS-BHK) and polyoma-transformed (PY-BHK) cells express two-

Abbreviations used: GlcNAc, N-acetylglucosamine; Man, mannose; MES, 2-[N-Morpholino]ethanesulfonic acid.

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fold higher levels of the N-linked antenna with the sequence [GlcNAcβ(1,6)Manα(1,6)Man] than do the parental nontransformed BHK cells. This particular oligosaccharide branch also contains almost all of the N-linked poly-N-acetylactosamine sequences expressed on these cells [2]. It appears, therefore, that transformation by either a DNA papovavirus or an RNA tumor virus causes an almost identical and specific change in N-linked oligosaccharide biosynthesis. These results explain (at a structural level at least) in part the original observations by Warren, Glick, and colleagues [3,4] that transformation of a number of cell types by either viral or chemical means results in an increase in the size of fucose-containing cell surface glycopeptides (reviewed in [5]).

By what mechanism(s) are the N-linked oligosaccharides of BHK cells specifically altered by Rous sarcoma and polyoma virus transformation? The simplest possibility is that the glycosyltransferase responsible for the biosynthesis of the [GlcNAcβ(1,6)Man] branch, UDP-GlcNAc:α-D-mannoside β(1,6)N-acetylglucosaminyltransferase V (Fig. 1), has a greater activity in the transformed cells. The greater GnT V-specific activity could be caused by an increased number of copies of the enzyme in the cells after virus transformation. Alternatively, transformation could cause the GnT V molecules to increase their rates of product formation, possibly by some posttranslational activation such as phosphorylation. But for either of these hypotheses to be correct, the effect on the number of copies of enzyme molecules or on translated enzyme activation would have to be specific for only one glycosyltransferase; otherwise, additional effects on N-linked oligosaccharide expression would be observed. Another explanation to account for the oligosaccharide structural changes observed upon transformation is that the topology of GnT V molecules in the Golgi apparatus is somehow altered. Since the template for oligosaccharide biosynthesis is the ultrastructure of the Golgi itself, an alteration in the position of the enzyme or its accessibility to the nascent oligosaccharide chains travelling through the Golgi could conceivably explain differences in glycosylation patterns, even if the specific activity of the enzyme was relatively unaltered after transformation. It is also conceivable that alterations in the rate of transfer of the oligosaccharide chains through the portion of the Golgi containing GnT V could influence increases in particular cell surface oligosaccharides. Presently, no means are available to test these possibilities adequately.

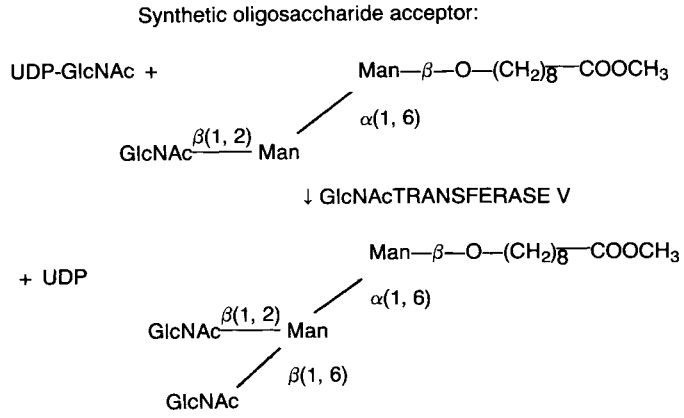

Fig. 1. Reaction scheme of GnT V using the synthetic oligosaccharide acceptor.

To determine if the specific activity of GnT V is increased in polyoma-transformed cells, Kobata and co-workers assayed all N-acetylglucosaminyl-transferases present in PY-BHK and BHK cells and showed that only GnT V had an altered activity in the transformed cells: PY-BHK cells had about a twofold increase in specific activity compared to BHK cells [6]. Therefore, although the increase of GnT V in the PY-BHK cells was modest, this enzyme appeared to be the only one affected by transformation.

Rous sarcoma virus is an RNA tumor virus (in contrast to polyoma virus which is a DNA virus) and the length of its nucleic acid component is less than that of the polyoma virus. As discussed, however, RH-BHK cells showed an increase in the "6-branch" structures after transformation, similar to that increase observed for PY-BHK cells. We have focused on testing whether GnT V-specific activity is increased in RS-BHK cells.

We sought to develop a rapid and precise assay for this enzyme that could be used to measure accurately kinetic parameters and that could ultimately be used in the purification of the enzymes. In collaboration with Dr. O. Hindsgaul of the Department of Chemistry, University of Alberta, a synthetic trisaccharide with an 8-carbon hydrophobic tail (scheme depicted in Fig. 1) was produced and characterized [7]. The trisaccharide corresponded to the structure which we predicted that GnT V most likely recognizes, since the enzyme does not appear to distinguish between biantennary and triantennary oligosaccharides containing the [GlcNAcβ(1,4)Manα(1,3)] branch. The hydrophobic aglycone of the acceptor allows it to bind avidly to pellicular C18 columns, commonly used for high-performance liquid chromatography (HPLC) sample cleanup. With this assay, the transfer of UDP-[^{3}H]-GlcNAc to the acceptor can be easily monitored by applying the enzyme incubation to the C18 column, washing to remove the radiolabeled UDP-GlcNAc and its breakdown products, and then eluting the radiolabeled product with methanol [8].

Experiments quickly demonstrated that both BHK and RS-BHK cells transferred GlcNAc to the trisaccharide, forming a single tetrasaccharide product [9]. The enzymatic transfer was not inhibited by 20 mM EDTA, a characteristic of GnT V [6,8,10]. The tetrasaccharide that corresponds to the predicted GnT V product was also synthesized and characterized, and the enzymatic product was shown by NMR analysis to be identical to this synthetic tetrasaccharide [9]. Other experiments [8] showed that GnT V could be measured in mouse lymphoma BW5147 cells, and that a lectin-resistant variant cell line, PHA^R 2.1, which lacks [GlcNAcβ(1,6) Man] sequences [10], has no GnT V activity.

Preliminary studies suggested that the specific activity of GnT V was about twofold higher in RS-BHK cells compared to BHK cells. As the conditions of the assay were optimized in terms of pH, detergent concentration, substrate concentration, and as breakdown of reactants and products was eliminated by the inclusion of specific inhibitors in the assay, the difference in specific activity of GnT V could be observed to be about fourfold in the RS-BHK cells.

As a first step in answering the question of how this increase in GnT V-specific activity is caused by virus transformation, we compared the kinetic properties and pH optima of the enzymes in sonicates of RS-BHK and BHK cells to determine if there was any evidence for an enzyme in the transformed cells with different catalytic properties. The conclusion from the results of these experiments is that the GnT V activities in RS-BHK and BHK sonicates are indistinguishable in terms of their simple kinetic properties.

EXPERIMENTAL PROCEDURES

Materials

UDP-GlcNAc, Triton X-100, and ADP were from Sigma (St. Louis, MO). UDP-[^{3}H]-GlcNAc was from ARC (St. Louis, MO) or synthesized in our laboratory (M. Shoreibah and M. Pierce, manuscript submitted). Pellicular C18 columns (Bond-elute, Analytichem International, Harbor City, CA) were used to bind the synthetic oligosaccharides.

Assay of GnT V Activity

The assay conditions were essentially identical to those described earlier [8]. Optimal assay conditions were determined to require a final concentration of 0.5% Triton X-100 at pH 6.0. Unless noted, the assays described in this report were performed by drying the following reagents under reduced pressure in a 0.75-ml plastic microfuge tube: UDP-[^{3}H]-GlcNAc, 100 nmol, about 10^6 cpm (10 cpm/pmol); GnT V acceptor, 20 nmol; ADP, 20 nmol; N-acetyl-*D*-glucopyranosylamine (β-hexosaminidase inhibitor), 50 nmol. Cells were grown on 10-cm plates as described [2], harvested, washed with PBS, and sonicated in about 2:1 v/v of 0.1 M MES, pH 6.0. After disruption with a probe sonicator, the final concentration of protein was about 20 mg/ml as measured by the microBradford method [11]. Ten microliters of 1.0% Triton X-100 in MES buffer were added to the assay tube containing the dried reactants and the walls of the tube were rinsed to insure complete dissolution of the reactants. Ten microliters of sonicate was then added to make a final volume of 20 μl, and the contents of the tube was mixed by repetitive pipetting. The tube was incubated for 2 hr at 37°C.

The sample was diluted with water and applied to a centrifuge filter device that contained about 0.1 ml of Dowex-1-formate. The device was centrifuged, and the filtrate, from which about 90% of the unused sugar-nucleotide was removed by the Dowex resin, was applied to the pellicular C18 column that had been activated with methanol and equlibrated in water. The column was washed with about 20 ml of water until the eluant contained only background radioactivity. The product was then eluted into scintillation vials with methanol, usually about five 1-ml fractions. Scintillation fluor was added to the vials and radioactivity measured.

RESULTS

The GnT V activities in BHK and RS-BHK cells were initially compared by assaying the enzymes over the pH range from 5 to 8. Figure 2 demonstrates that the enzymes from both cells showed a similar broad optima from pH 5.5 to 7.5, with the highest activity at pH 6. It is also apparent that when the enzymes were assayed under optimal conditions in which the substrate concentrations were saturating, the breakdown of substrates and product was inhibited, and the pH and detergent concentrations were optimized; the difference in specific activity of GnT V between the two cell types was about fourfold. When the enzymes were measured at higher pH, as was done in the previous studies on this enzyme [6,10], the difference in specific activities was decreased.

The saturation kinetics for UDP-GlcNAc was investigated next. Figure 3, panels 1 and 2, shows that similar kinetics for this substrate were observed with GnT V from both BHK and RS-BHK cells. Another separate determination of the apparent K_m for RS-BHK cells for UDP-GlcNAc was performed (data not shown), and when the apparent

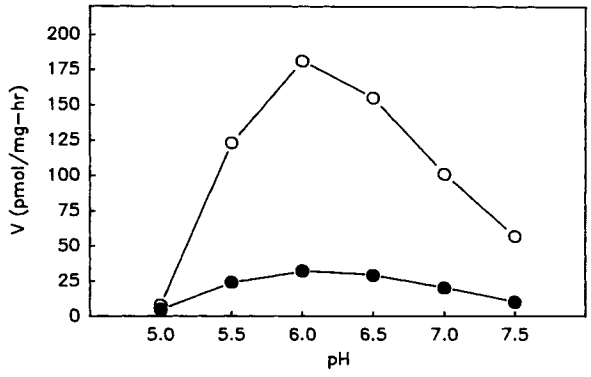

Fig. 2. Activity of GnT V in BHK and RS-BHK cells as a function of pH. Cells were sonicated and assayed under optimal conditions for activity as described, except that the pH of the MES buffer was varied from pH 5 to pH 7.5. (●), BHK cells; (○), RS-BHK cells.

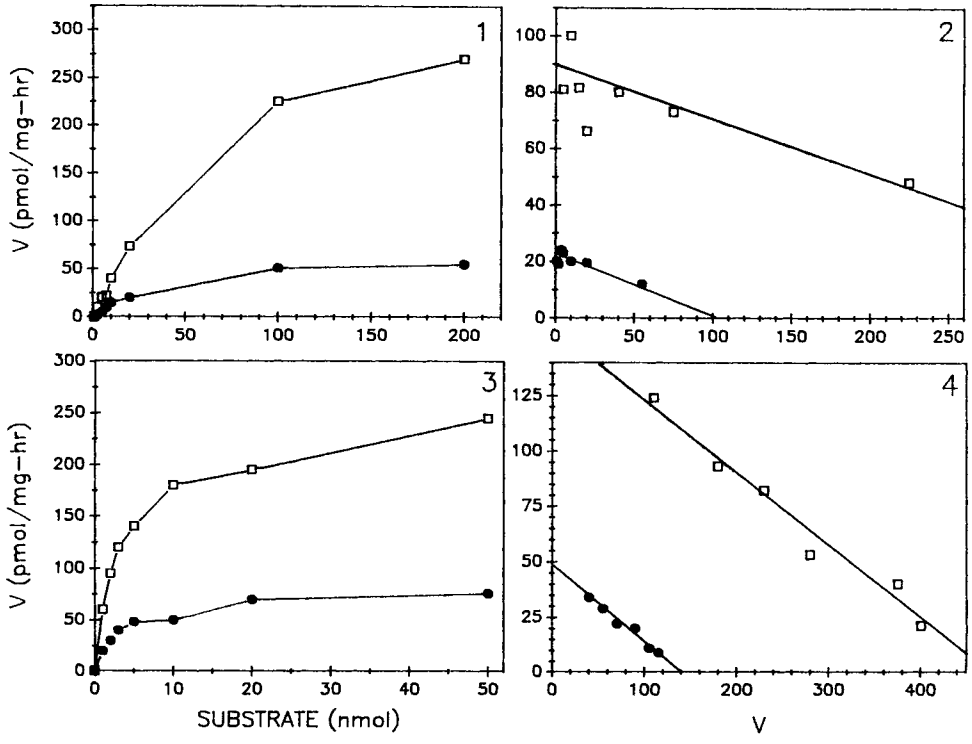

Fig. 3. Activity of GnT V in BHK and RS-BHK cells as a function of substrate concentration. Cells were assayed under optimal conditions as described. (●), BHK cells; (□), RS-BHK cells. **Panels 1** and **2**, UDP-GlcNAc; **panels 3** and **4**, trisaccharide acceptor.

K_m values from both sets of data were averaged, the K_m was calculated to be 244.4 ± 39.6 μM. The correlation coefficients (r values) for these calculations were 0.83 and 0.98. The apparent average K_m for this substrate and the BHK GnT V enzyme was 250.8 ± 14 μM, r = 0.81 and 0.85. Thus, the K_m values for UDP-GlcNAc for GnT V from both cells were essentially identical.

A similar experiment determined the saturation kinetics and K_m values for the synthetic oligosaccharide acceptor for GnT V from BHK and RS-BHK cells. Figure 3, panels 3 and 4, shows that the saturation kinetics for the acceptor were similar for GnT V from the two cell types and that the apparent K_m value for the RS-BHK enzyme was 161.4 μM (r = 0.99), while that for the BHK enzyme was 175.5 μM (r = 0.97). Taken together, these results demonstrate that the GnT V enzymes from BHK and RS-BHK cells appear to be very similar in their apparent K_m values for both UDP-GlcNAc and synthetic acceptor.

DISCUSSION

Although there are many direct examples of changes in glycosyltransferase-specific activities during development and after cell transformation, there is really no information on the mechanisms by which any of these changes in activity are actually achieved. Do these changes occur by mechanisms that regulate the number of genes transcribed or the amount of message transcribed, or is glycosyltransferase activity also regulated in situ by posttranslational modification of the enzymes? The only documented mechanism of the modulation of a glycosyltransferase activity by the binding of an effector molecule appears to be the inhibition of N-acetylactosamine synthesis by the interaction of α-lactalbumin with GlcNAcβ(1,4)galactosyltransferase [12]. Do other specifier proteins exist in the Golgi apparatus and regulate glycosyltransferase activity by direct interaction with specific enzymes?

One system that could allow a detailed examination of the regulation of a glycosyltransferase activity is the specific increase in GnT V activity that occurs when BHK fibroblasts are transformed by Rous sarcoma or polyoma viruses. After developing a synthetic trisaccharide acceptor for GnT V and optimizing assay conditions, the initial question to address was whether the GnT V from the transformed cells displayed a similar pH profile to the enzyme from the parental BHK cells. The results show that the pH profiles are similar, with an optimum at pH 6.0 for both enzymes, suggesting that the RS-BHK cells do not express another GnT V activity with a significantly different profile.

If the K_m constants of the enzymes from both cells for either substrate differed significantly, then this result would provide evidence that the RS-BHK cells expressed a GnT V with altered catalytic properties. The results show that when using cell sonicates as a source for the enzymes, no significant difference could be observed in the K_m values of the enzymes for either substrate. Although we are not as yet studying purified enzymes, no evidence suggests that the kinetic properties of the majority of GnT V activity expressed in RS-BHK differ from those expressed in BHK cells.

We are presently conducting experiments to determine directly if factors present in RS-BHK can stimulate GnT V activity in BHK cell sonicates. If significant differences in the kinetic or physical properties of the purified enzymes from the two cells cannot be demonstrated, then the specific increase in GnT V activity caused by infection of the cells by Rous sarcoma virus must be explained either by a regulatory step occurring

before actual translation of active enzyme, by an altered topology of the enzyme in the Golgi, or by a mechanism that selectively allows nascent oligosaccharide chains to remain adjacent to GnT V molecules for longer periods of time during their transit through the Golgi.

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