INCORPORATION OF [^{14}C]-2 DEOXY-D-GLUCOSE INTO THE LIPIDS OF NORMAL CELLS AS COMPARED TO VIRUS-TRANSFORMED CELLS

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Summary - [^{14}C]-2 deoxy-D-glucose is incorporated into the glycolipids of both normal and transformed cells. The chromatographic patterns of [^{14}C]-2 deoxy-D-glucose labeled lipids differ markedly in oncornavirus and herpes simplex virus-transformed cells as compared to normal and virus-infected but not transformed cells. Deoxyglucose-labeled lipids with intermediate chromatographic mobility were enriched in normal and virus-infected but not transformed cells. Studies with a murine sarcoma virus-infected cell line which is temperature-sensitive for transformation indicated that the altered chromatographic pattern of [^{14}C]-2 deoxy-D-glucose labeled lipids was related to the expression of the transformed phenotype.

Viral transformation results in the reduction in the more complex glycolipids, usually with a concomitant increase in simpler ones (1-3). Both decreases in glycolipid glycosyl transferases (4-6) and increases in glycolipid hydrolases have been observed in transformed cells (5, 7). Studies in this laboratory have demonstrated that the glucose or mannose analogue, 2 deoxy-D-glucose[1], is incorporated intact into both lipids and proteins and that at low levels it is not inhibitory to cellular growth (8, 9). On the basis of the previous studies we set forth to determine if [^{14}C]-dglc was a useful precursor for differentiation between the synthesis of glycolipids in normal as compared to oncornavirus- or herpes simplex virus-transformed cells. The results show that the

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[1] The abbreviations used are: dglc, 2 deoxy-D-glucose; NRK, normal rat kidney cells; NRK (MSV-MLV), murine sarcoma virus-murine leukemia virus transformed NRK cells; NRK (MSV-1b), MSV-infected NRK cells which are temperature-sensitive for transformation; Wis, normal Wistar rat embryo cells; 78A1, MSV-MLV-transformed Wistar rat cells; LSH, normal hamster embryo fibroblast cells; 333, herpes simplex virus type 2 transformed LSH cells.

pattern of incorporation of dglc into the glycolipids of normal as compared to the transformed cells differ markedly.

MATERIALS AND METHODS

Cells and culture conditions: The cell lines used in this study were: NRK, normal rat kidney cells; NRK (MSV-MLV), murine sarcoma virus-murine leukemia virus-transformed and producing NRK cells; NRK (MSV-1b), a non-producing NRK cell line with a rescuable MSV genome which is temperature-sensitive for transformation (10); Wis, normal Wistar rat embryo cells; MLV-Wis, Wis cells chronically infected with and producing MLV; 78A1, MSV-MLV-transformed and producing Wistar rat cells; 333, herpes simplex virus type 2-transformed hamster cells, the generous gift of Dr. F. Rapp; and LSH, normal hamster embryo fibroblast cells. Cells were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum (v/v), antibiotics and 0.2 μCi/ml (3.8 μM) [^{14}C]-dglc (New England Nuclear, Boston, Mass.) where indicated. Permissive and nonpermissive temperatures for NRK (MSV-1b) were 39 and 33°C, respectively.

Cell harvesting and fractionation: Cells were washed three times with cold saline and then scraped into a small volume of saline and sonicated. The homogenate was centrifuged at 100,000 g for 45 min. The particulate fraction was washed three times with cold saline. The final pellet was resuspended in 0.2 ml of H_2O for lipid extraction.

Lipid extraction and analysis: The particulate fraction was extracted twice with chloroform-methanol (2:1, v/v) and twice with chloroform-methanol (1:2, v/v). The lipid was chromatographed on silica gel plates (Q5, Quantum Industries, Fairfield, N.J.) in isopropanol-NH_4OH-H_2O (7:2:1, by vol.). Radioactivity was detected by autoradiography (Kodak No-Screen X-ray film). The plates were scraped in 1-cm bands from origin to solvent front and radioactivity was measured by scintillation spectrometry (8). In order to identify the nature of the radioactivity incorporated into lipid, lipid plus 4 μmoles of dglc was hydrolyzed in 0.05 N H_2SO_4 at 100°C for 20 min to release [^{14}C]-dglc. The reaction mixture was neutralized with excess $BaCO_3$. Chromatography and detection of nonradioactive and radioactive dglc were as previously described (8). For pronase treatment, the lipid fraction was taken to dryness and resuspended in 1 ml of H_2O plus 0.005 ml of pronase (10 mg/ml) in 1 M Tris-HCl, pH 8.0. The mixture was incubated at 37°C for 5 hr and then chromatographed on a silica gel plate as described above. Mild alkaline methanolysis was performed by the method of Lester and Steiner (11). The lipid samples were Folch-partitioned as described by Radin (12). Protein was estimated by the method of Lowry et al. (13).

RESULTS AND DISCUSSION

To establish that the [^{14}C]-dglc-labeled material extracted from the washed high speed pellet was lipid, the following criteria were used: (i) solubility in chloroform-methanol, 2:1 and/or 1:2 (v/v), (ii) resistance to pronase, (iii) non-dialyzability, and (iv) chromatographic properties on silicic acid thin layer plates. In addition, chromatography of [^{14}C]-dglc-labeled cytosol material did not reveal a significant level of

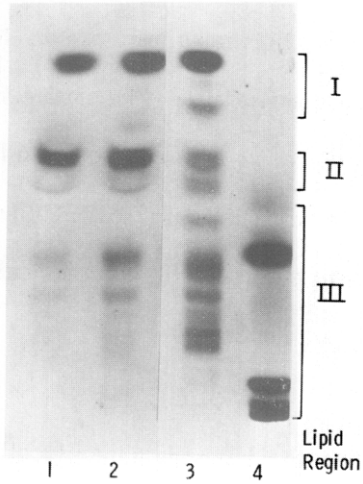

FIGURE 1. Autoradiogram of a thin layer chromatogram of [^{14}C]-dglc-labeled lipids from Wis and MLV-Wis cells and [^{14}C]-dglc-labeled lipids and cytosol fraction from 78A1 cells. Lane 1 - Wis lipid; 2 - MLV-Wis lipid; 3 - 78A1 lipid; 4 - 78A1 cytosol. Cells were grown in [^{14}C]-dglc medium for 2 days. They were harvested and lipid extracted and chromatographed as described in the text.

components with Rf values comparable to those of the lipid components (see Fig. 1).

The incorporation of [^{14}C]-dglc into the lipids of normal and oncornavirus-transformed cells was examined. Figure 1 is an autoradiogram of a one-dimensional chromatogram of [^{14}C]-dglc-lipids of normal Wis cells (lane 1), MLV-infected but not transformed Wis cells (lane 2), and MSV-MLV-transformed Wis cells (lane 3). Approximately the same amount of radioactivity was applied to each lane. All the lipid components were resistant to mild alkaline methanolysis, suggesting that they are glycosphingolipids and all the [^{14}C] radioactivity of these components was released as dglc upon mild acid hydrolysis. Chromatography of a significant portion of the cytosol [^{14}C] radioactivity (Fig. 1, lane 4) reveals little, if any, components with Rf values comparable to the [^{14}C]-dglc-lipids. The pattern of incorporation of [^{14}C]-dglc radioactivity into non-transformed and transformed cell lipids differs markedly. In calculating the distribution of radioactivity, the [^{14}C]-dglc-lipids were grouped into three major areas of radioactivity (see Fig. 1), based on chromatographic mobility and therefore presumably on the complexity of the oligosaccharide moiety. Table 1 shows the percent

distribution of the [^{14}C] components illustrated in Figure 1. Normal and MLV-infected, but not transformed, Wis cells have approximately the same distribution of radioactivity, thus indicating that virus replication alone is not sufficient to induce altered [^{14}C]-dglc-lipid patterns. However, the percentage of [^{14}C]-dglc in the lipids of intermediate chromatographic mobility in transformed cells is dramatically decreased while those of both higher and lower mobility are increased.

TABLE I

Percent Distribution of [^{14}C]-Dglc into the Glycolipids of Normal, MLV-Infected and MSV-MLV-Transformed Wistar Rat Cells

Lipid region	% radioactivity			Normal/ MLV	Normal/ MSV-MLV
	Normal	MLV	MSV-MLV-transformed		
I	34	39	49	0.9	0.7
II	42	36	7	1.2	6.0
III	23	24	44	1.0	0.5

Percent distribution of [^{14}C]-dglc-labeled lipids illustrated in Fig. 1.

TABLE II

Percent Distribution of [^{14}C]-Dglc into the Glycolipids of NRK (MSV-1b) Cells Grown at Permissive and Nonpermissive Temperature

Lipid region	% radioactivity		Nonpermissive/ permissive
	Nonpermissive	Permissive	
I	22.5	57.4	0.4
II	50.6	8.5	6.0
III	27.0	34.0	0.8

NRK (MSV-1b) cells were grown at the permissive, 39°C, or nonpermissive, 33°C, temperature for 2 days in medium supplemented with [^{14}C]-dglc. The cells were harvested and lipid extracted and analyzed as described in the text. Lipid regions are as indicated in Fig. 1.

In order to establish more clearly whether or not the differences in these lipid patterns were related to transformation, a well characterized, cold-sensitive transformation mutant was examined. The distribution of [^{14}C]-dglc into glycolipids of NRK (MSV-1b) at nonpermissive temperature, i.e., normal phenotype, as compared to NRK (MSV-1b) cells at permissive temperature, i.e., transformed phenotype, are shown in Table 2. The decrease in the percent radioactivity in lipid region II in the lipid sample from cells grown at the permissive temperature indicates that the altered [^{14}C]-dglc-lipid labeling is related to the transformed phenotype. NRK cells and the like cells infected with wild-type MSV-MLV have [^{14}C]-dglc-lipid patterns similar to those shown in Table 2 for the mutant with normal and transformed phenotypes, respectively.

Transformed cells may deplete the medium of glucose more rapidly than normal cells. Since dglc and glucose are competitive for entry into the cell, a consequence of lowered glucose in the medium could be increased uptake of [^{14}C]-dglc. In order to rule out the possibility that the differences in [^{14}C]-dglc-lipid chromatographic patterns were due to differences in the intracellular levels of dglc, experiments were undertaken in which the total amount of [^{14}C]-dglc/mg protein were similar in normal and transformed cells. This was achieved by growing cells in [^{14}C]-dglc supplemented medium which was changed twice daily for four days. Under these conditions the intracellular concentrations of dglc in NRK and MSV-MLV NRK cells were similar and the [^{14}C]-dglc-lipid chromatographic patterns were comparable to the patterns obtained from the like cells grown for four days in the same medium. In related studies we have observed that addition of ten times more dglc to the medium, i.e., 40 μM dglc, had no effect on the relative chromatographic patterns of [^{14}C]-dglc labeled lipids from normal and transformed cells. As with the frequent medium change, these results also suggest that the observed differences in dglc lipid patterns are not primarily related to the internal concentration of dglc but are reflective of a difference(s) in dglc lipid metabolism.

To examine the possibility that similar changes to those observed with oncorna-

TABLE III

Percent Distribution of [^{14}C]-Dglc into the Glycolipids of Normal and Herpes Simplex Virus-Transformed Hamster Cells

Lipid region	% radioactivity		Normal/ transformed
	Normal	Transformed	
I	22	27	0.8
II	51	25	2.0
III	27	49	0.6

LSH and 333 cell lines were grown in [^{14}C]-dglc supplemented medium for 5 days (one change of medium). The cells were harvested and lipid extracted and analyzed as described in the text. Lipid regions are as indicated in Fig. 1.

virus-transformed cells occur in DNA virus-transformed cells, the incorporation of [^{14}C]-dglc into the lipids of herpes simplex virus type 2 transformed hamster cells and normal hamster cells was studied. The results (Table 3) indicate that the relative incorporation of [^{14}C]-dglc into the glycolipids with intermediate chromatographic mobility are also reduced in herpes simplex virus-transformed cells.

Thus, the incorporation of [^{14}C]-dglc into glycolipid is altered in both DNA and RNA virus-transformed cells as compared to normal cells. The difference in glycolipid labeling pattern may reflect quantitative differences in the enzymes involved in dglc-glycolipid metabolism and/or it may be a consequence of a differential response of the enzymes of normal and transformed cells to a dglc-substituted substrate. In this regard, quantitative differences between glycosyl transferases of normal and transformed cells have been reported (4-6) and a qualitative difference between sialyl transferase of normal and transformed cells has also been observed (6). Cell-free studies of dglc-lipid metabolism are planned which should help clarify the basis of the difference in dglc-lipid metabolism between normal and transformed cells. In addition to the basic consideration

of glycolipid synthesis in normal versus transformed cells, the results of this study suggest that [^{14}C]-dglc-lipid patterns may be useful as a marker for transformation.

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