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Glycolipid Content of Vesicular Stomatitis Virus Grown in Baby Hamster Kidney Cells

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Vesicular stomatitis virions grown in baby hamster kidney (BHK-21-F) cells were found to contain hematoside (neuraminosyl-galactosyl-glucosyl-ceramide). This ganglioside, which was the only detectable glycolipid in the virion, is also the only glycolipid found in significant amount in BHK-21-F cells. Approximately 87% of the total neuraminic acid in the virion was found to be linked to protein and 13% to lipid.

The presence of carbohydrate linked to proteins in the viral envelope has been firmly established with many different types of viruses, including vesicular stomatitis virus (VSV; 2, 12, 14). Carbohydrate has also been found in glycolipids in enveloped virus particles; in the parainfluenza virus SV5 grown in MDBK cells, one-third of the carbohydrate in the viral envelope is present in glycolipids and two-thirds in glycoproteins (8, 9). Comparison of the glycolipids of SV5 grown in cells whose membranes have different glycolipid compositions indicated that the neutral glycolipids of the host cell membrane are incorporated into the virus. On the other hand, the neuraminic acid-containing glycolipids, the gangliosides, were not found in the SV5 virion, presumably due to the action of the viral enzyme neuraminidase (8-10). Neuraminidase is not a component of VSV, and we report here the presence in VSV virions of the same ganglioside that is present in the membrane of the baby hamster kidney (BHK-21-F) cells in which the virus was grown.

BHK-21-F cells were grown in plastic petri dishes as described previously (4). Cells were inoculated with the Indiana strain of VSV at a multiplicity of 10 to 50 plaque-forming units per cell, and, after an adsorption period of 90 min, reinforced Eagle's medium (1) was added. Approximately 17 hr after infection, the supernatant medium was harvested. Virus was purified by the procedure described for SV5 (6) which involved low-speed centrifugation to remove cellular debris, ammonium sulfate precipitation, and repeated equilibrium centrifugation in a preformed 10 to 40% potassium tartrate gradient. Two visible bands are found in such gradients. The lower band contains cellular material as well as some VSV particles. The upper band, which was used for all chemical analyses, was found by electron microscopy to contain VSV particles and no visible cellular debris; on polyacrylamide gel electrophoresis this material showed the typical VSV pattern of three major polypeptides (5, 13) with no evidence of contamination by cellular proteins.

Three virus samples, containing 50, 40, and 36 mg of protein, and a sample of uninfected BHK-21-F cells, prepared as described previously (8) and containing 330 mg of protein, were used for lipid analyses. Glycolipids were isolated from lyophilized samples and identified as described previously in detail (8). These procedures consisted of chloroform-methanol-water extraction, partition of the extract into an aqueous and an organic phase, and identification of the gangliosides in the aqueous phase by thin-layer chromatography. The gangliosides were quantitated by determination of the neuraminic acid content by the method of Warren (15). Protein was determined by the method of Lowry et al. (11).

The glycolipid of BHK-21 cells has been found to consist almost entirely of hematoside (neuraminosyl-galactosyl-glucosyl-ceramide; 3, 8). Figure 1 shows that VSV virions grown in BHK-21 cells also contain hematoside as the only glycolipid present in significant amount. The hematoside content of the virions (440 μg/100 mg of protein) was higher than that of the whole cell (100 μg/100 mg of protein). This is to be expected since the virus buds from the cell membrane and hematoside is located predominantly in the plasma membrane of the BHK-21-F cells (8). These results indicate that the glycolipid composition of VSV is similar to that of the host cell plasma membrane, a result similar to

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that found with the parainfluenza virus SV5 (7, 8).

Neuraminic acid is known to be a constituent of the glycoprotein of VSV (2, 12). We determined the relative amounts of this substance in the glycoprotein and glycolipid in VSV virions grown in BHK-21-F cells and found that per 100 mg of viral protein, 680 μg of neuraminic acid (87%) was protein-bound and 100 μg (13%) was lipid-bound.

In addition to demonstrating that the glycolipid composition of VSV resembles that of the host cell, the present results establish that neuraminic acid is a constituent of glycolipid as well as glycoprotein in the virion. The finding of neuraminic acid in VSV virions and its absence in SV5 virions grown in the same cell type (8) support the conclusion that the absence of this substance in SV5 virions is due to the incorporation of the viral neuraminidase into the regions of the host cell plasma membrane which eventually become the viral membrane (8–10).

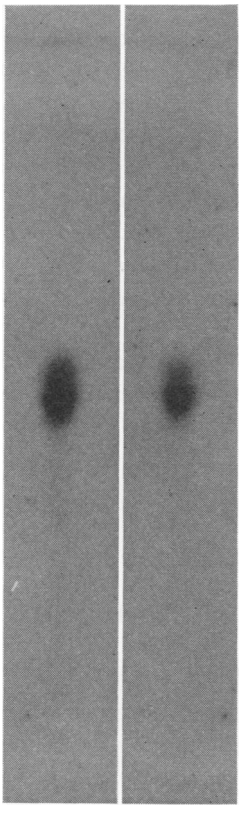

FIG. 1. *Thin-layer chromatogram of gangliosides from BHK-21-F cells (left) and from VSV grown in these cells (right). The only ganglioside present is hematoside (neuraminosyl-galactosyl-glucosyl-ceramide) which appears as a double spot. Silica gel plate (Merck, Germany). Solvent: chloroform-methanol-water (60:35:8). Stain: Bial's reagent.*

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