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Mosquito Cells Infected with Vesicular Stomatitis Virus Yield Unsialylated Virions of Low Infectivity

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Vesicular stomatitis virus propagated in and released from *Aedes albopictus* cells had the normal complement of viral proteins; the glycoprotein contained carbohydrate but no sialic acid. These virions had markedly reduced hemagglutinating activity and exhibited a very high ratio of physical particles to infectious virus. In vitro sialylation of vesicular stomatitis virions grown in mosquito cells resulted in a 100-fold increase in both infectivity and hemagglutination titers to levels approaching those of virus grown in BHK-21 cells. These experiments provide an example of host-controlled modification of viral infectivity.

Vesicular stomatitis (VS) virus is an RNA-containing, enveloped rhabdovirus which grows in a wide variety of cultured cells of mammalian and arthopod origins (1, 3, 15). Infectiom of mosquito cell lines with either VS virus or arboviruses (togaviruses) generally results in a low-titered, noncytolytic, persistent infection (2, 4, 6, 8, 12). The mechanism which regulates this low-grade infection of mosquito cells is unknown. Neither interferon production nor the presence of other antiviral factors could be demonstrated in cultures of infected *Aedes aegypti* or *Aedes albopictus* cells (2, 6, 8, 12).

We have previously demonstrated that the sialic acid content of VS viral glycoprotein is, in part, responsible for initiation of viral infection (9) and that these neuraminic acid residues are required for maximal attachment of VS virions to cells (10). Since Warren (14) had observed that many species of the arthopod phylum lack sialic acid, we decided to investigate the possibility that the low infectivity of VS virus cultivated in *A. albopictus* cells is due to failure of the viral glycoprotein to be sialylated.

The *A. albopictus* cell line originally derived by Singh (11) was kindly supplied by S. M. Buckley of the Yale Arbovirus Research Unit. Cultures were maintained at 25 C and passed by scraping and suspending cells in mosquito culture medium (GIBCO, Grand Island, N.Y.) which was supplemented with 2% inactivated fetal calf serum. Plaque-purified VS virus (Indiana serotype) grown in BHK-21 cells was used to infect *A. albopictus* cell cultures at a multiplicity of infection of ~10 PFU/cell. After adsorption of virus to cells for 1 h at 37 C, mosquito cell growth medium was added, and the infected cells were incubated at 37 C for 24 h, at which time radioactive isotopes were added. Released virions were collected 72 h postinfection, and VS-B virions were purified as described previously (5). Cultivation of VS virus in BHK-21 cells, polyacrylamide gel electrophoresis, and enzymatic treatment of virions with *Vibrio cholerae* neuraminidase and BHK-cell-derived sialyl transferase were performed essentially as reported elsewhere (9). Infectivity levels were determined by plaque assay on monolayers of L cells, and hemagglutination titrations with goose erythrocytes were performed as described previously (9) except that virions were not grown in the presence of bovine plasma albumin.

Figure 1 shows the electrophoretic profile of proteins extracted from purified VS-B virions grown in *A. albopictus* cells (VS_{albo}virus) which were labeled with [^{3}H]glucosamine and [^{14}C]-labeled amino acids. All five VS viral proteins (L, G, N, NS, and M) are present in VS_{albo} virions. In addition, [^{3}H]glucosamine label is found in those regions of the gel to which the viral glycoprotein and glycolipids migrate. Moreover, treatment of VS_{albo} virions with *V. cholerae* neuraminidase, which resulted in removal of 15 to 20% of the sugar label from BHK-grown (VS_{BHK}) virus (9), had no effect on the [^{3}H]glucosamine label in either the G protein or the glycolipids (data not shown).

Since VS virions grown in *A. albopictus* cells incorporate label poorly, sialic acid content of these VS_{albo} virions was measured by the thiobarbituric acid assay (13). Table 1 shows that

neuraminic acid could not be detected in VS_{albo} virus or in uninfected and infected *A. albopictus* cells. In contrast, both BHK-21 cells and VS_{BHK} virions contained considerable amounts of sialic acid. In addition, attempts to detect sialyltransferase activity in either infected or uninfected *A. albopictus* cells were unsuccessful as measured by transfer of [^{14}C]sialic acid from the nucleotide sugar to desialylated fetuin.

In view of this evidence that VS_{albo} virions lack detectable neuraminic acid, the presence of which on viral glycoprotein is essential for efficient attachment of virions to cells (10), we explored the possibility that in vitro sialylation of these VS_{albo} virions would result in increased infectivity. For comparison, VS_{BHK} virions, which already contain sialic acid, were also subjected to identical treatment. Table 2 demonstrates the low infectivity of VS_{albo} virions compared to high-plaque titers of equivalent numbers of VS_{BHK} virions. This difference in infectivity of virus grown in the two cell types is more accurately depicted as the ratio of physical particles to infectious units, which was consistently 10^3 to 10^4 times greater for VS_{albo} virus than it was for VS_{BHK} virus. Although neuraminidase desialylation markedly reduced infectivity of VS_{BHK} virions, treatment with neuraminidase had no effect on the already low infectivity of VS_{albo} virus.

Proof that absence of sialic acid is responsible for the low infectivity of VS_{albo} virus required confirmatory evidence that enhanced infectivity would result from inserting sialic acid residues into the VS_{albo} viral glycoprotein. To this end VS_{albo} virions were incubated with partially purified BHK-cell sialyl transferase and CMP-[^{14}C]sialic acid. This procedure resulted in incorporation of ~2,000 molecules of sialic acid per VS_{albo} virion, about 90% of which were covalently linked to the glycoprotein. This degree of sialylation increased infectivity of VS_{albo} virions almost 100-fold, from a level of 1 PFU/65,000 virions to 1 PFU/780 virions (Table 2). Removal of incorporated sialic acid from VS_{albo} virus by neuraminidase resulted in reduction of infectivity to almost the original low levels. By comparision, sialyl transferase did not enhance infectivity of VS_{BHK} virions unless they had been previously desialylated with neuraminidase (9).

The hemagglutinating activities of VS_{albo} and VS_{BHK} virions were also compared to provide a measure of their differential capacity to adsorb to cell surfaces. Equivalent amounts of virions grown in the two cell types were tested by the hemagglutination assay using goose erythrocytes buffered at pH 6.1 (10). VS_{BHK} virions exhibited considerable hemagglutinating activity, which was markedly reduced by exposure to neuraminidase (Table 2). In sharp contrast, the intrinsic capacity of VS_{albo} virions to agglutinate goose erythrocytes was quite limited, but in vitro sialylation of VS_{albo} virions endowed them with almost as much hemagglutinating activity as that of VS_{BHK} virions. This enhanced hemagglutinating activity of VS_{albo} virions could be reversed by treatment with neuraminidase. Residual hemmagglutinating activity of VS_{albo} virions and VS_{BHK} virions extensively treated with neuraminidase indicates that neuraminic acid residues are not solely responsible for hemagglutination. However, this residual hemagglutinating activity of VS_{albo} and

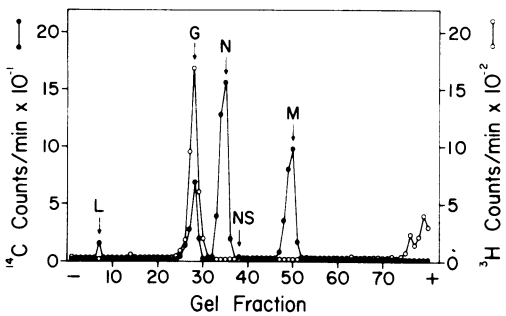

FIG. 1. *Electropherogram of VS viral proteins grown in A. albopictus cells. VS virus grown in A, albopictus cells was labeled for 48 h with [^{3}H]glucosamine (2 μCi/ml) and ^{14}C-labeled amino acids (1 μCi/ml). Released virions were purified by differential and rate zonal centrifugation in 0 to 40% sucrose gradients. Virus suspension (~150 μg) was made 1% with respect to sodium dodecyl sulfate and 2-mercaptoethanol and boiled for 2 min. Samples were subjected to electrophoresis for 8 h at 5 mA/gel on 7.5% polyacrylamide gels. The gels were processed as described previously (9). Positions of viral proteins were determined by parallel electrophoresis of VS_{BHK} virion marker proteins.*

TABLE 1. *Sialic acid content of cells and purified VS virions grown in A. albopictus and BHK cells*[a]

Material	Sialic (μg)	Protein (mg)
VS_{albo} virions	<0.1	1.87
VS_{BHK} virions	10.8	0.99
A. albopictus cells	<0.1	24.4
BHK-21 cells	36.0	17.7

[a] Sialic acid content was determined by the Warren method (13), after mild acid hydrolysis at pH 1.5 for 2 h at 80 C. Protein concentrations were measured by the Lowry procedure (7).

TABLE 2. *Comparative infectivity and hemagglutination titers of VS virus grown in A. albopictus and BHK-21 cells*

Virus	Treatment[a]	Infectivity[b] (PFU/ml)	Particle-to-PFU ratio[c]	Hemagglutination[d]	
				U	U/mg of protein
VS_{albo} virions	None	1.2×10^8	65,000	16	400
	Neuraminidase	1.1×10^8	71,000	16	359
	Sialylation	9.5×10^7	780	2,048	50,000
	Sialylation, then neuraminidase	1.6×10^8	49,000	32	735
VS_{BHK} virions	None	4.0×10^9	17	4,096	97,238
	Neuraminidase	9.6×10^8	7,300	8	181
	Sialylation	4.5×10^9	16	4,096	94,194
	Sialylation, then neuraminidase	8.7×10^8	8,300	8	157

[a] Purified VS virions which had been labeled with ^{3}H-labeled amino acid were sialylated by CMP-[^{14}C]sialic acid in the sialyl transferase for 3 h at 37 C or desialylated by 50 U of neuraminidase for 1 h at 37 C, as previously described (9, 10).

[b] Plaque assays were performed on monolayers of L cells.

[c] Estimated from viral protein determinations.

[d] Optimal pH of hemagglutination titrations for VS virions grown in *A. albopictus* and BHK cells was determined to be pH 6.1.

neuraminidase-treated VS_{BHK} virions was abolished by exposure to trypsin, indicating the importance of the viral glycoprotein in hemagglutination (data not shown).

The possibility that the low infectivity of unsialylated VS_{albo} virions is due to aggregates of virions which are subsequently dispersed by in vitro sialylation was examined by electron microscopy of preparations negatively stained with phosphotungstic acid. Suspensions of VS_{albo} virions showed no tendency to form aggregates either before or after in vitro sialylation. Moreover, protruding spikes appeared quite normal on VS_{albo} virions, as well as on VS_{BHK} virions treated with neuraminidase.

The results of these experiments provide additional evidence that neuraminic acid present on VS viral glycoprotein significantly enhances infectivity and adsorption of VS virus to host cells. These data also serve to confirm our previous hypothesis that host cell modification of the efficiency of VS viral infectivity and adsorption is mediated by in vivo sialylation of the VS viral glycoprotein (9, 10). The absence of sialic acid and sialyl transferase in *A. albopictus* cells appears to provide at least a partial explanation for the reduced infectivity and perhaps persistent infection of VS virus in mosquito cells. However, it is unlikely that the absence of sialic acid is the only factor regulating low-grade infection of mosquito cells by VS virus. Artsob and Spence (1) observed defective T particles in mosquito cells infected with VS virus, the presence of which could also interfere with replication of infectious B virions.

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LITERATURE CITED

1. Artsob, H., and L. Spence. 1974. Growth of vesicular stomatitis virus in mosquito cell lines. Can. J. Microbiol. **20**:329-336.
2. Artsob, H., and L. Spence. 1974. Persistent infection of mosquito cell lines with vesicular stomatitis virus. Acta Virol. **18**:331-340.
3. Buckley, S. M. 1969. Susceptibility of the *Aedes albopictus* and *Aedes aegypti* cell lines to infection with arboviruses. Proc. Soc. Exp. Biol. Med. **131**:625-630.
4. Davey, M. W., and L. Dalgarno. 1974. Semliki Forest virus replication in cultured *Aedes albopictus* cells: studies on the establishment of persistence. J. Gen. Virol. **24**:453-463.
5. Emerson, S. U., and R. R. Wagner. 1972. Dissociation and reconstitution of the transcriptase and template activities of vesicular stomatitis B and T virions. J. Virol. **10**:297-309.
6. Libikova, H., and S. M. Buckley. 1971. Studies with Kemerovo virus in Singh's *Aedes* cell lines. Acta Virol. **15**:393-403.
7. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
8. Peleg, J. 1969. Inapparent persistent virus infection in continously grown *Aedes aegypti* mosquito cells. J. Gen. Virol. **5**:463-471.
9. Schloemer, R. H., and R. R. Wagner. 1974. Sialoglycoprotein of vesicular stomatitis virus: role of the neura-

minic acid in infection. J. Virol. **14**:270–281.
10. Schloemer, R. H., and R. R. Wagner. 1975. Cellular adsorption function of the sialoglycoprotein of vesicular stomatitis virus and its neuraminic acid. J. Virol. **15**:882–893.
11. Singh, K. R. P. 1967. Cell cultures derived from larvae of *Aedes albopictus* (Skuse) and *Aedes aegyptic* (L). Curr. Sci. **36**:506–508.
12. Stollar, V., and T. E. Shenk. 1973. Homologous viral interference in *Aedes albopictus* cultures chronically infected with Sindbis virus. J. Virol. **11**:592–595.
13. Warren, L. 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**:1971–1975.
14. Warren, L. 1963. The distribution of sialic acids in nature. Comp. Biochem. Physiol. **10**:153–171.
15. Zee, Y. C., A. J. Hackett, and L. Talens. 1970. Vesicular stomatitis virus maturation sites in six different host cells. J. Gen. Virol. **7**:95–102.