

INHIBITION OF VESICULAR STOMATITIS VIRUS INFECTION BY SPIKE GLYCOPROTEIN

Evidence for an Intracellular, G Protein-requiring Step

DOUGLAS K. MILLER and JOHN LENARD

From the Department of Physiology and Biophysics, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854

ABSTRACT

In an assay measuring virus-directed RNA synthesis, infection of BHK cells by a standard test dose of vesicular stomatitis virus (VSV) was inhibited by ultraviolet light-irradiated *wt* VSV and by *ts* 045, one of a number of thermolabile, temperature-sensitive G protein mutants of VSV. After heat treatment for 1 h at 45°C, the thermolabile mutants were no longer able to inhibit the VSV infection. In contrast, the thermolabile M protein mutant *ts* G31 and the nonthermolabile G protein mutant *ts* 044 could still inhibit the test VSV dose. Thus, the presence of G protein in its native conformation was necessary for inhibition of infection. There was little difference in the binding to cells or the internalization to a trypsin-resistant state of *ts* 045 or *wt* VSV before and after heat treatment, and there was no evidence of specific saturable receptors on the cell surface. None of the irradiated virions at concentrations that gave maximal inhibition of infection could prevent binding of infectious VSV to, or internalization by, BHK cells. The G protein-specific inhibition, therefore, did not occur at the cell surface but must have occurred at some intracellular site, which has been suggested to be the lysosome. The lysosomal inhibitor chloroquine, when added with the infecting virus, completely inhibited VSV infection at all multiplicities of infection tested, and it gave 50% inhibition when added 1.5 h after infection. The possible importance of the lysosome in the intracellular pathway of infection is discussed.

KEY WORDS vesicular stomatitis virus ·
temperature-sensitive mutants · G protein ·
thermolability · virus binding · virus
internalization · chloroquine

The spike glycoprotein G of vesicular stomatitis virus (VSV)¹ has been shown to be essential to the

infectivity of VSV in BHK cells: both removal of the G protein by proteolysis (1) and coincubation of the cells with purified G protein in vesicular form (13) inhibit viral infection. It has been widely accepted, but not directly shown, that the importance of the G protein rests in its ability to enable VSV to attach to cells (2). In this study, we have

¹ *Abbreviations used in this paper:* VSV, vesicular stomatitis virus; PBS, Dulbecco's phosphate-buffered saline; MEM, Eagle's minimal essential medium; BES, *N,N*-

bis(2-hydroxyethyl)-2-aminoethane sulfonic acid; SFV, Semliki Forest virus; BSA, bovine serum albumin; FCS, fetal calf serum; m.o.i., multiplicity of infection.

focused on the cellular locus at which G acts by using thermolabile, temperature-sensitive G protein mutants of VSV. These mutants are called thermolabile because the purified mutant virions, grown at 31°C, are inactivated by incubation at the nonpermissible temperature (39–45°C). It has previously been shown that such thermal inactivation of G protein mutants (complementation group V) of VSV occurs with specific changes in the properties of the mutant G protein, suggesting conformational alterations (11). We now report evidence that the loss of infectivity in the heat-treated, thermolabile G protein mutants is neither a function of an inability of the virus to bind to the cells, nor an inability to transfer to trypsin-insensitive sites but rather occurs as the result of their inability to perform a specific function at some internal site.

MATERIALS AND METHODS

Cell and Virus Growth

BHK-21F cells were grown in glass bottles and passed twice weekly in Eagle's minimal essential medium (MEM) with Earle's salts supplemented with 10% calf serum (Grand Island Biological Co., Grand Island, N. Y.), 400 U/ml penicillin G, and 100 µg/ml streptomycin. For individual inhibition or binding experiments, the BHK cells (10–40 passages) were grown to confluence in 35-mm Corning culture dishes (Corning Glass Works, Science Products Div., Corning, N. Y.).

Wt VSV, Indiana serotype, was prepared in confluent Corning roller bottles (Corning Glass Works) (2×10^6 cells) by inoculation at a multiplicity of infection (m.o.i.) of 1 and growth for 1 d in 40–50 ml of serum-free medium at 37°C. The VSV complementation group V (G protein) mutants (*ts* 045, *ts* 057, *ts* 0110, *ts* 044, and *tl* 17) and the complementation group III (M protein) mutant (*ts* G31) were similarly inoculated and grown at 31°C. Defective, interfering particles were generally not seen in virus grown under these conditions. The virions were harvested by centrifugation at 12,000 rpm for 2 h at 5°C in a Sorvall GSA rotor (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.) (7.5×10^4 g-min) and were purified by ultracentrifugation in 15–60% sucrose gradients for 90 min at 25,000 rpm in an SW 27 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) (5.4×10^6 g-min) followed by dialysis against Dulbecco's phosphate-buffered saline (PBS).

Virions to be tested for inhibitory activity were inactivated by irradiation for 5 min with 2,500 ergs/sec/cm² at 254 nm with a germicidal lamp. This irradiation dose was found to be the minimum dose that effectively inactivated viral transcriptase activity, which agrees very closely with the recent findings of Weck et al. (18). Irradiated virions were tested for inhibitory activity before and after heat treatment for 1 h at 45°C.

Spikeless Particles

About 500 µg of VSV was pelleted and resuspended in 2 ml of pronase (1 mg/ml) in PBS. After a 90-min incubation at 37°C (12), the digest was layered on a linear 20–40% potassium tartrate gradient and centrifuged for 90 min at 40,000 rpm at 5°C in a

Beckman SW41 rotor (Beckman Instruments, Inc.). The spikeless particle band was collected and dialyzed against PBS.

RNA Inhibition Assay

To measure the amount of inhibition of VSV RNA production, confluent monolayers of BHK-21F cells (10^6 cells per monolayer) were preincubated for 60 min at 37° or 5°C, with or without inhibitor, in 0.5 ml of a 1:1 mixture of PBS and MEM containing a final concentration of 50 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid (BES) (pH 7.0) and 100 µg/ml of bovine serum albumin (BSA). A standard m.o.i. of *wt* VSV (e.g., 10 PFU/cell)² was added in 0.5 ml of MEM containing 20% fetal calf serum (FCS) and 20 µg/ml actinomycin D,³ and incubation was continued at 37°C for an additional 45–60 min. The cells were washed once, and 1 ml of MEM containing 10% FCS, 10 µg/ml actinomycin D, and 3–7 µCi/ml [³H]uridine (5 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) was added. After a final 4–5-h incubation at 37°C, the cells were washed and solubilized in SDS. The RNA was precipitated in TCA, filtered, and counted for radioactivity as previously described (13). The amount of inhibition was determined by comparison with counts obtained from a standard curve obtained by infecting plates of cells at various m.o.i.s in the absence of any inhibitor (13).

Virus Binding

Radioactively labeled *wt* VSV and *ts* 45 were prepared by growing viruses in unlabeled methionine-free medium containing [³⁵S]methionine (5 µCi/ml, >1,000 Ci/mmol, Amersham Corp.). The most highly labeled viruses were prepared in unlabeled methionine-free medium containing 10% of the normal amounts of amino acids, [³⁵S]methionine as described above, 60 µCi/ml of ³H-amino acids (15–60 Ci/mmol, Amersham Corp.), and 60 µCi/ml [³H]uridine (45 Ci/mmol). Binding of the labeled viruses to the cells was determined by incubation of serial dilutions of the viruses in 0.25 ml of MEM containing 50 mM BES and 100 µg/ml BSA for periods up to 4 h at 5° or 37°C. At the desired time, the unbound virus was removed by aspiration, and the cells were washed twice with cold PBS and removed from the plate by incubation for 10 min at 5°C with 2 ml of trypsin solution (80 µg/ml PBS) with gentle shaking. The cells were centrifuged, washed once with PBS, and solubilized in 1 ml of 1% SDS. Aliquots of the trypsin supernate and the pellet solution were counted for radioactivity to determine the trypsin-sensitive and the trypsin-insensitive components of the total cell-associated radioactivity.

RESULTS

Differential Inhibition of Infection by Heat-inactivated Mutants

A test VSV infection of BHK cells, as measured by the RNA assay, was completely inhibited by preincubation with ultraviolet light (UV)-irradiated *wt* VSV, with 50% inhibition occurring at 0.5–1 µg *wt* VSV/ 10^6 cells (Fig. 1). The greater the test

² This will be referred to as "test VSV," in contrast to the UV-irradiated VSV used as an inhibitor.

³ The generous gift of Merck & Co., Rahway, N. J.

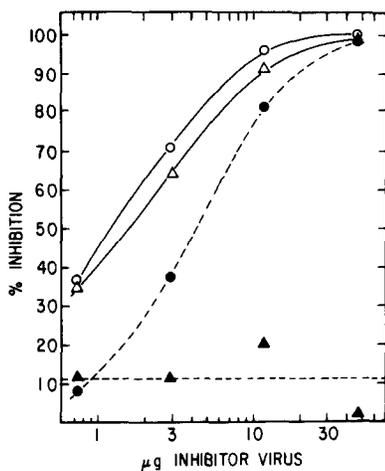


FIGURE 1 Differential inhibition of VSV RNA production by UV-irradiated *wt* VSV and *ts* 45 before and after heat treatment. Viruses were UV irradiated, heated, and assayed for their ability to inhibit VSV RNA production. Test VSV was 10 PFU/cell. Cells were preincubated with inhibitors for 60 min at 37°C and incubated for 6 h with 5 μ Ci/ml [3 H]uridine. \circ , *wt* VSV, no heat treatment; \bullet , *wt* VSV, after heat treatment; Δ , *ts* 45, no heat treatment; \blacktriangle , *ts* 45, after heat treatment.

dose of VSV, the greater the concentration of inhibitor required for 50% inhibition (not shown). UV-irradiated *ts* 45, a temperature-sensitive G protein mutant, inhibited the *wt* VSV infection almost as well as the irradiated *wt* VSV. After heat treatment, however, the *ts* 45 completely lost its inhibitory capability, while the heat-treated, irradiated *wt* VSV lost only ~50–70% of its inhibitory capacity (Fig. 1). It has previously been shown that similar heat treatment of infectious mutant virions results in loss of infectivity, a property called thermolability (11). When the inhibitory capacity of several other thermolabile G protein mutants (*ts* 57, *ts* 110, and *tl* 17) was tested, all showed good inhibition before heat treatment, whereas essentially no inhibitory activity remained after heat treatment (Table I). In contrast, after a similar incubation, both the nonthermolabile G protein mutant *ts* 44 and the thermolabile M protein mutant *ts* 31 (11) inhibited the VSV infection as well as the heat-treated *wt* VSV did. Thus, inhibition of VSV infection by irradiated virions requires a G protein of normal conformation. The loss of inhibitory activity upon heating is not due simply to a mutation in the G protein or to the thermolability of an inhibitory virus, but to the specific combination of both.

Ts 45 and *wt* VSV were tested before and after heat treatment for their ability to inhibit the precipitation by specific anti-G antiserum (13) of [35 S]methionine-labeled G protein extracted from *wt* VSV. After heat treatment, the *ts* 45 was as able to inhibit binding of the labeled G protein as was *wt* VSV (data not shown). Thus, the conformational change induced by heat treatment is not reflected in a significant change in antibody-binding properties.

Effect of Preincubation Time

It was not necessary to preincubate the BHK cells with UV-irradiated virus to obtain inhibition of infection. When the inhibitory virus was added at the same time as the infecting *wt* VSV dose, a similar inhibition curve was obtained (Fig. 2). The

TABLE I
Inhibition of UV-irradiated VSV infection by temperature-sensitive mutants

Virus	TS complementation group	Thermolability	μ g Viral protein necessary for 50% inhibition*	
			- Δ	+ Δ
WT VSV	—	—	0.6–1.5	4.4
TS 045	V	+	1.5	$\gg 47$
TS 057	V	+	2.4	$\gg 21$
TS 0110	V	+	3.8	$\gg 35$
TL 17	V	+	1.2	$\gg 50$
TS 044	V	—	0.6	1.2
TS G31	III	+	0.9	2.4

* Determined from inhibition curves as in Fig. 1.

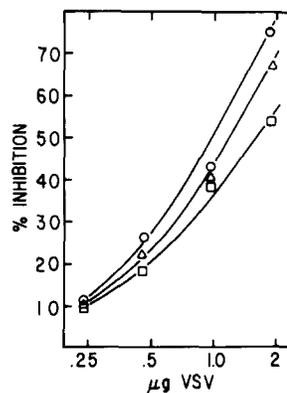


FIGURE 2 Effect of preincubation time. BHK cells were preincubated for 0 (\square), 20 (Δ), or 40 (\circ) min with the indicated amount of UV-irradiated *wt* VSV before addition of test VSV at 10 PFU/cell for 1 h at 37°C. Cells were washed and incubated in 5 μ Ci/ml [3 H]uridine as described in Materials and Methods.

increased inhibition with preincubation continued for up to 2 h, with most of the increase occurring within 1 h. As shown in Fig. 2, the concentration necessary for 50% inhibition decreased by 40% after 40 min of preincubation.

Inhibition is Long Lasting

To test the persistence of inhibition after removal of the inhibitor, BHK cells were preincubated for 60 min at 37°C with 2 µg/plate of UV-irradiated *wt* VSV, washed, and placed in growth medium free of virus. When the cells were examined for their inhibition of a subsequent test VSV challenge, it was found that inhibition was maintained for at least 24 h (Fig. 3). One group of inhibited cells, incubated in similar growth medium containing 1 µg/ml of actinomycin D to prevent interferon production (7), showed similar results (Fig. 3).

Binding of *wt* VSV and *ts 45* to BHK Cells

Because decreased binding of heat-inactivated *ts 45* to BHK cell surfaces may have been the cause of its reduced inhibition, the amount of adsorption of UV-irradiated labeled *wt* VSV and *ts 45* to cells was measured. Both viruses bound continuously to BHK cells both before, and after, heat treatment without obvious saturation for at least 4 h (Fig. 4A). Live (non-UV-treated) *wt* VSV and *ts 45* bound to the BHK cells at a rate indis-

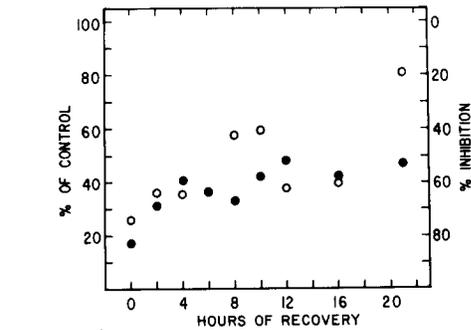


FIGURE 3 Long lasting effect of inhibition. BHK cells were preincubated with 2 µg of UV-irradiated *wt* VSV/10⁶ cells for 60 min at 37°C. The cells were washed and incubated in growth medium for 0–21 h with (●) or without (○) 1 µg/ml actinomycin D. At the indicated times, cells were incubated at 37°C with test VSV (2 PFU/cell) for 45 min, washed, and incubated with 3 µCi/ml [³H]uridine for 4 h.

tinguishable from that of irradiated virus for up to 1 h, but little additional binding occurred after that time (data not shown). Binding of the irradiated viruses was not saturable by increasing the amount added up to a concentration of 12 µg/plate; a similar proportion of the added virus bound regardless of concentration (Fig. 5A and B). The time course of binding was similar at each concentration (not shown). The total difference in percent bound was less than 40% for *wt* VSV and

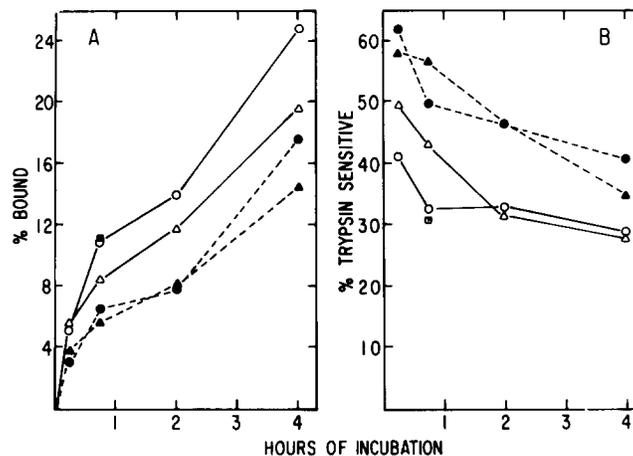


FIGURE 4 Time course of binding. BHK cells were incubated with 0.13 µg of ³H- and ³⁵S-labeled *wt* VSV and *ts 45* (160,000 cpm/µg) as described in Materials and Methods. (A) Percent of the total radioactivity added to the cells that was bound at the indicated time. (B) Percent of the radioactivity bound to the cells that was removed by trypsin treatment. ○, UV-irradiated *wt* VSV, no heat treatment; □, live *wt* VSV, no heat treatment; ●, UV-irradiated *wt* VSV, after heat treatment; △, UV-irradiated *ts 45*, no heat treatment; ▲, UV irradiated *ts 45*, after heat treatment.

ts 45 over a 300-fold concentration range. At the lowest concentration of added virus, roughly seven particles were bound per cell.⁴ The existence of specific receptors at a level of less than seven particles per cell could not be determined. The heat-treated virions bound slightly less well than the untreated ones, and spikeless particles of *wt* VSV bound even less well (Fig. 5*A* and *B*).

Internalization of *wt* VSV and *ts 45*

The rate of internalization of virus was measured by trypsin treatment at 5°C of the cells after virus adsorption. This treatment had the dual purpose of removing cells from the plate and releasing cell surface-bound virus. Under the conditions employed, over 80% of the virus bound at 5°C could be released by a 10-min trypsin treatment. The trypsin-soluble counts represent the minimum counts present at the cell surface. Not all trypsin-insensitive counts are necessarily internal counts, because some virus at the cell surface may not have been removed.

The amount of trypsin-sensitive virus was independent of the amount of bound virus over a 300-fold range, indicating that virus became internalized in proportion to the amount bound (Fig. 5*C* and *D*). Heat-treated *wt* VSV and *ts 45* were both more trypsin-sensitive than the nonheat-treated virus. Spikeless particles were still more trypsin sensitive. The amount of virus that was trypsin sensitive decreased with time of incubation at 37°C, as would be expected from an internalization process (Fig. 4*B*).

In the experiments described above, the amount of trypsin-sensitive virus was a function of both the rate at which virus was bound and the rate at which the bound virus was internalized. To measure the latter rate only, *ts 45*, both with and without heat treatment, was bound to BHK cells for 1 h at 5°C. The cells were washed, and the plates were then incubated at 37°C in growth medium. The results of this experiment (not shown) indicate that: (*a*) about half of the bound virus was released almost immediately after warming; (*b*) two-thirds of the bound virus that was not released became trypsin insensitive within 15 min; (*c*) the remaining one-third of the bound virus remained trypsin sensitive without change for up to 75 min after warming; (*d*) there was no differ-

ence between heat-treated and nonheat-treated *ts 45*. Similar results were obtained with concentrations of *ts 45* in the binding medium ranging from 0.2 to 5.5 µg virus/plate.

Differential Inhibition of Binding and Infection

The observation that a relatively constant proportion of added virus was always bound suggested that there were no saturable receptors for binding of *wt* VSV and/or *ts 45* up to the maximum concentration employed. To show conclusively that the binding and internalization of virus were not differentially affected by heat inactivation, radioactively labeled infectious *wt* VSV was used to measure both binding and RNA production. BHK cells were preincubated for 1 h at 37°C with nonradioactive, irradiated *wt* VSV or *ts 45*, both with and without heat treatment. The labeled infectious *wt* VSV was then added to the cells, and the amount bound, the amount internalized, and the amount of viral RNA subsequently formed were determined using the same techniques described above. There was no inhibition of the radioactive VSV binding by any of the four unlabeled, irradiated virion preparations (Fig. 6*A*) and little change in the rate of internalization as measured by trypsin insensitivity (Fig. 6*B*). In contrast, the RNA production showed the previously observed differential inhibition (Fig. 6*C*). Thus, neither binding nor internalization of infectious virions is inhibited by levels of irradiated virions that effectively inhibit viral RNA synthesis.

Inhibition of VSV Infection by Chloroquine

The results suggested that the site of inhibition of VSV infection was not at the cell surface but at some internal site. It is commonly thought that internalized particles or molecules ultimately fuse with lysosomes, after which they are either destroyed or recycled (16). A commonly used inhibitor of lysosomal function is chloroquine, a lipid-soluble, weakly basic quinoline analogue that has been found to accumulate in lysosomes, inhibiting their proteases and raising their pH (14). When BHK cells were preadsorbed with a test VSV inoculum in the cold, washed, and incubated at 37°C in medium containing actinomycin D, [³H]uridine, and 100 µM chloroquine, inhibition of viral RNA production was essentially complete (Fig. 7). Other amines, including methylamine, propylamine, and isopropylamine, gave complete

⁴ Assuming 5.8×10^{-16} g/virion; calculated from data of Bishop and Smith (2), Table 6, using Lowry protein, with BSA as a standard.

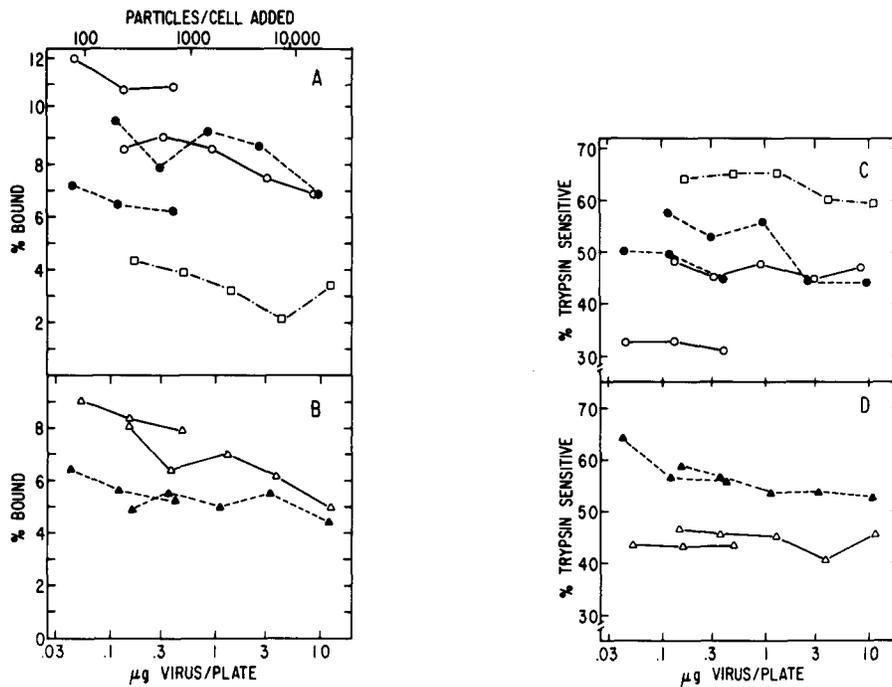


FIGURE 5 Binding of UV-irradiated *wt* VSV (A) and *ts* 45 (B) to BHK cells as a function of concentration. Binding was for 45 min at 37°C. Percent of the radioactivity bound to the cells that was removed by trypsin treatment for *wt* VSV (C) and *ts* 45 (D). Results of two experiments (points from each connected by lines). ○, *wt* VSV, no heat treatment; □, *wt* VSV, spikeless particles; ●, *wt* VSV, after heat treatment; △, *ts* 45, no heat treatment; ▲, *ts* 45, after heat treatment.

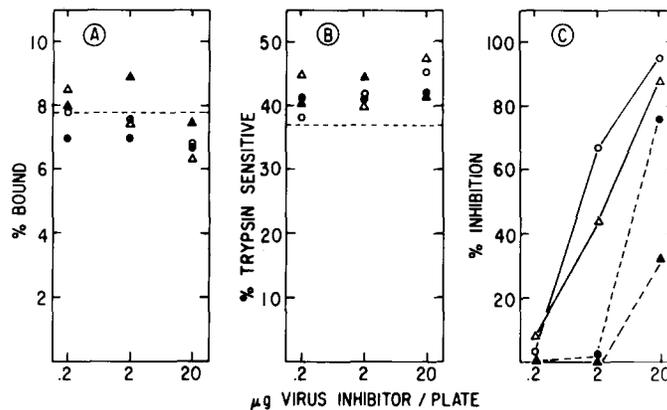


FIGURE 6 Inhibition of binding and RNA production of live ³⁵S- and ³H-labeled *wt* VSV by UV-irradiated *wt* VSV and *ts* 45 before and after heat treatment. Radioactive *wt* VSV (160,000 cpm/μg, 0.06 μg/plate, 3–4 PFU/cell) was added to BHK monolayers that had been incubated for 60 min at 37°C with UV-irradiated *wt* VSV and *ts* 45 at the indicated concentrations. Plates were incubated with radioactive test virus for an additional 60 min at 37°C and analyzed as described in Materials and Methods for binding and RNA inhibition. (A) Percent of total radioactivity added to the cells that was bound. (B) Percent of the bound radioactivity that was removed by trypsin. (C) Percent inhibition of RNA synthesis. Broken lines indicate values in the absence of inhibitors. ○, UV-irradiated *wt* VSV, no heat treatment; ●, UV-irradiated *wt* VSV, after heat treatment; △, UV-irradiated *ts* 45, no heat treatment; ▲, UV-irradiated *ts* 45, after heat treatment.

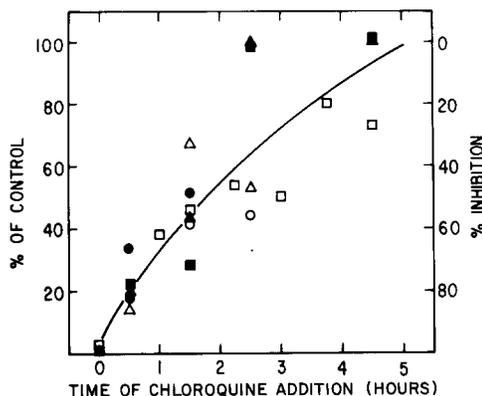


FIGURE 7 Time dependence of chloroquine inhibition of VSV-directed RNA synthesis. BHK monolayers were incubated for 60 min at 5°C with live *wt* VSV at the PFU/cell indicated below. The cells were washed and incubated at 37°C in medium containing actinomycin D and [³H]uridine as described in Materials and Methods. At the indicated times, 100 μM chloroquine was added to the medium and maintained until the end of the 5-h assay incubation. Results of two experiments. Δ, 1.8 PFU/cell; □, 3 PFU/cell; ○, 7.2 PFU/cell; ▲, 29 PFU/cell; ■, 115 PFU/cell; ●, 350 PFU/cell.

inhibition of infection at ~25 mM concentrations. The chloroquine inhibition was evident even when chloroquine was added several hours after exposure to test virus; 50% inhibition occurred when chloroquine was added 1.5 h after the initiation of infection (Fig. 7). The time dependence of chloroquine inhibition was independent of m.o.i. in the range of 1.8 to 350 PFU/ml. Thus, despite the fact that there is a greater production of viral RNA by cells infected at higher m.o.i. (13) the rate at which the infectious virions reach the chloroquine-sensitive step is the same. This suggests that at all multiplicities the viruses go through the same chloroquine-sensitive step and that this step cannot be bypassed.

In contrast to the inhibition of viral RNA production shown here, host cell RNA and protein synthesis were inhibited <20% over a 24-h period, as measured by [³H]uridine and [³⁵S]methionine incorporation into TCA-precipitable material. Preincubation of the test VSV with 100 μM chloroquine before addition to the BHK monolayers containing chloroquine-free medium did not appreciably inhibit viral RNA production. RNA polymerase activity of detergent-disrupted VSV in an *in vitro* assay system was unaffected by concentrations of chloroquine up to 100 μM, suggesting that chloroquine was not acting directly

upon the process of viral RNA synthesis (A. T. Wilson and J. Lenard, unpublished observation).

DISCUSSION

The rhabdovirus VSV and the alphaviruses Sindbis and Semliki Forest virus (SFV) have been the focus of many previous studies of the mechanism of penetration and uncoating of enveloped RNA viruses. Electron microscopic (3, 17) and immunological (5) evidence has shown that both groups enter by viropexis, an endocytotic mechanism. Helenius et al. (9) have suggested that the overall process of SFV infection involves binding of virus to cell surface receptors, transfer of virus to coated pits, internalization into coated vesicles, and transport to storage vesicles that ultimately fuse with primary lysosomes. At some point, the viral nucleocapsid exits from the lysosome, presumably by fusion of the viral envelope with the lysosomal membrane. This process bears close resemblance to the process of internalization of low-density lipoproteins, certain peptide hormones, and other molecules with specific cell surface receptors (8), all of which ultimately collect in lysosomes.

The present study suggests both differences and similarities in the mechanisms of entry of VSV and SFV. Helenius et al. (10) have shown by direct binding and various immunochemical criteria that initial binding of SFV to the host cell surface occurs between the spike glycoproteins and the cell receptors that are involved with the histocompatibility antigens. These receptors are saturable, and the binding of intact SFV can be inhibited by purified spike rosettes. Our results show, in contrast, no evidence either by direct binding or by inhibition of binding for saturable binding sites for VSV on BHK cells. It seems clear that if a saturable receptor is involved in VSV infection of BHK cells, it cannot be distinguished from the remaining very large population of receptor sites that have similar affinity for VSV. In all cases, the amount of RNA formed in our assay bears a direct correspondence to that predicted on the basis of the amount of total virions bound and the experimentally determined particle/PFU ratio, that is, "productive" (RNA-producing) virus is a constant proportion of total bound virus. Comparison of the binding results presented here with those of Helenius et al. (10) is difficult because of differences in cell types and the valencies of the bound ligand. At the highest concentration tested, we observed 1.5 μg of virus binding per 10⁶ cells, with little effect on the subsequent binding of labeled

test virus (Figs. 5 and 6). This is equivalent to 3×10^3 receptors, if each virion is bound by one receptor, or 4.5×10^6 receptors if all the G protein molecules in each bound virion interact with separate receptors. The number of binding sites found by Helenius et al. (10) ($<3 \times 10^5$ SFV binding sites per cell) falls in the range delimited by these two values.

The lack of specificity of the BHK cell surface for VSV is also indicated by the observation that many VS virions fall off the cell surface while others remain but are not internalized. Flamand and Bishop (6) have shown that, in 12 cycles of adsorption with BHK cells, the particle/PFU ratio is identical for those VS virions that bind to BHK cells, for those that do not bind at all, and for those virions that fall off after binding. Hence, there can be no preferential selection by the cells of virions that lead to a productive infection from those that do not. There is no evidence to suggest that there is an innate difference between virions that will become productive and those that will not. It seems more likely that the existence of a particle/PFU ratio of >1 for VSV⁵ is due not to inherent differences within the particles themselves, but rather, at least in part, to the inefficiency of the cellular uncoating process. Virions may be lost at numerous stages in the infection process: release after binding, failure to internalize, destruction within lysosomes (4).

Consideration of the outlined sequence of events suggests the importance of the viral surface at two steps in uncoating: interaction, first, with receptors on the outer surface of the plasma membrane and, second, with the inner part of the lysosomal membrane, where virus and cell membrane may fuse to effect release of the genome into the cytoplasm. In the intervening steps of viral transport between the cell surface and lysosome, it is possible that the particle, bound within a cellular vesicle, could be swept along without regard to the specific nature of its outer surface. Because the lysosome is the most probable site of chloroquine and aliphatic amine inhibitory action on VSV and SFV infection (9), the two viruses may share a common pathway at this point in the infectious process. Interestingly, irradiated *wt* VSV inhibits infection

⁵ The *wt* VSV and *ts* 45 used in this study had a particle/PFU ratio of 20 and 40, respectively (see footnote 4).

by Sindbis and SFV virus at concentrations very similar to those that effectively inhibit VSV infection (D. K. Miller and J. Lenard, unpublished observation). Whether the VSV inhibition of alphaviruses is dependent upon a conformationally active G protein molecule is under investigation.

The authors are indebted to Ms. M. Kmetz for her excellent technical assistance.

This research was supported by National Institutes of Health grant AI 13002 and MSRP grant 27-9852 from the College of Medicine and Dentistry of New Jersey.

Received for publication 27 July 1979, and in revised form 15 October 1979.

REFERENCES

- BISHOP, D. H. L., P. REPIK, J. F. OBIESKI, N. F. MOORE, and R. R. WAGNER. 1975. Restitution of infectivity to spikeless vesicular stomatitis virus by solubilized viral components. *J. Virol.* **16**:75-84.
- BISHOP, D. H. L., and M. S. SMITH. 1977. Rhabdoviruses. In *The Molecular Biology of Animal Viruses*. Debi Nayak, Editor. Marcel Dekker, Inc. New York. 167-280.
- DAHLBERG, J. E. 1974. Quantitative electron microscopic analysis of the penetration of VSV into L cells. *Virology*. **58**:250-262.
- DALES, S. 1973. Early events in cell-animal virus interactions. *Bacteriol. Rev.* **37**:103-135.
- FAN, D. P., and B. M. SEFTON. 1978. The entry into host cells of Sindbis virus, vesicular stomatitis virus and Sendai virus. *Cell*. **15**:985-992.
- FLAMAND, A., and D. H. L. BISHOP. 1973. Primary *in vivo* transcription of vesicular stomatitis virus and temperature-sensitive mutants of five vesicular stomatitis virus complementation groups. *J. Virol.* **12**:1238-1252.
- FRIEDMAN, R. M. 1977. Antiviral activity of interferons. *Bacteriol. Rev.* **41**:543-567.
- GOLDSTEIN, J. L., R. G. W. ANDERSON, and M. S. BROWN. 1979. Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature (Lond.)*. **279**:679-685.
- HELENIUS, A., J. KARTENBECK, K. SIMONS, and E. FRIES. 1980. On the entry of Semliki Forest virus into BHK-21 cells. *J. Cell Biol.* **84**:404-420.
- HELENIUS, A., B. MOREIN, E. FRIES, K. SIMONS, P. ROBINSON, V. SCHIRRMACHER, C. TERHORST, and J. L. STROMINGER. 1978. Human (HLA-A and HLA-B) and murine (H-2K and H-2D) histocompatibility antigens are cell surface receptors for Semliki Forest virus. *Proc. Nat. Acad. Sci., U. S. A.* **75**:3846-3850.
- KELLER, P. M., E. E. UZGIRIS, D. H. CLUXTON, and J. LENARD. 1978. Aggregation and thermostability of some group V (G protein) and group III (M protein) mutants of vesicular stomatitis virus. *Virology*. **87**:66-72.
- MC SHARRY, J. J., R. W. COMPANS, and P. W. CHOPPIN. 1971. Proteins of vesicular stomatitis virus and of phenotypically mixed vesicular stomatitis virus-simian virus 5 virions. *J. Virol.* **8**:722-729.
- MILLER, D. K., B. I. FEUER, R. VANDERROEF, and J. LENARD. 1980. Reconstituted G protein-lipid vesicles from vesicular stomatitis virus and their inhibition of VSV infection. *J. Cell Biol.* **84**:421-429.
- OHKUMA, S., and B. POOLE. 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. *Proc. Nat. Acad. Sci., U. S. A.* **75**:3327-3331.
- PRINGLE, C. R. 1975. Conditional lethal mutants of vesicular stomatitis virus. *Curr. Top. Microbiol. Immunol.* **69**:85-116.
- SILVERSTEIN, S. C., R. M. STEINMAN, and Z. A. COHN. 1977. Endocytosis. *Annu. Rev. Biochem.* **46**:669-722.
- SIMPSON, R. W., R. E. HAUSER, and S. DALES. 1969. Viropexis of vesicular stomatitis virus by L cells. *Virology*. **37**:285-290.
- WECK, P. K., A. R. CARROLL, D. M. SHATTUCK, and R. R. WAGNER. 1979. Use of U.V. irradiation to identify the genetic information of vesicular stomatitis virus responsible for shutting off cellular RNA synthesis. *J. Virol.* **30**:746-753.