Role of Viral Envelope Sialic Acid in Membrane Fusion Mediated by the Vesicular Stomatitis Virus Envelope Glycoprotein

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ABSTRACT: Fusion of vesicular stomatitis virus (VSV) with cells and liposomes before and after treatment with neuraminidase was studied using the R18 dequenching assay. Desialylation of VSV significantly enhanced the extent of fusion with Vero cells but affected neither the pH dependence nor the binding of VSV to Vero cells. The enhanced fusion of asialo-VSV was observed both at the plasma membrane as well as via the endocytic pathway. Both VSV and asialo-VSV fused with liposomes made of neutral phospholipid, but only asialo-VSV fused with liposomes containing a 1:1 mixture of neutral and negatively charged phospholipid. To examine factors which contribute to the extent of fusion, we analyzed the various activation and inactivation reactions that take place as a result of low-pH triggering of VSV prebound to the target membrane. Lag times for the onset of fusion were similar for VSV and asialo-VSV, indicating that desialylation did not affect the activation reactions. However, exposure of VSV bound to target membranes at pH 6.5 for 400 s led to considerable inactivation, whereas little inactivation was seen after desialylation of VSV. These results are analyzed in terms of a model which allows us to determine which components of the overall fusion process are dominated by viral envelope sialic acid.

The envelope of vesicular stomatitis virus (VSV)[1] consists of a bilayer membrane with a single type of glycoprotein, the G-protein, which mediates attachment to the cell surface and induces pH-dependent fusion between viral and target membranes (Pal et al., 1987; Ohnishi, 1988). We have studied initial steps in VSV G-protein-mediated fusion with different target membranes using a lipid mixing assay based on relief of self-quenching of R18 upon fusion (Blumenthal et al., 1987; Puri et al., 1988; Grimaldi et al., 1988; Clague et al., 1990; Herrmann et al., 1990). In studies with cultured cells, we were able to distinguish between low-pH-induced fusion at the plasma membrane versus entry via the endocytic pathway. Studies of the mechanism of pH-triggered VSV fusion using rapid kinetics revealed a complex series of activation steps before lipid mixing takes place (Clague et al., 1990). On the basis of those data, we proposed a model, according to which pH-dependent viral fusion occurs when the envelope proteins undergo pH-activated conformational changes while the virus is appropriately bound to the target membrane. pH-dependent conformational transitions that occur while the virus is not in the proper apposition with respect to the target membrane will result in a failure to fuse, or "inactivation". If the rates of activation reactions leading to fusion are slow relative to those of the inactivation pathway, suboptimal extents will be reached, whereas rapid activation rates will yield optimal extents.

In order to gain further insight into the mechanism of VSV fusion, we examined the role of electrostatic and steric constraints which are known to be important barriers to membrane fusion (Blumenthal, 1987). Steric constraints can arise from integral membrane proteins, which extend out a considerable distance into the medium. This will prevent bilayers from coming together. Electrostatic repulsions due to proteins might also play a role. Theoretical calculations (Bell et al., 1984) and experimental evidence suggest that there is a strong nonspecific repulsive force between most cells, arising from electrostatic repulsion and from solvent exclusion from the hydrated polymers extending from their surfaces (steric stabilization). The negative charge on surface glycoproteins is due to terminal sialic acid residues. Removal of this residue by neuraminidase dramatically increases the aggregation potential of red blood cells in dextran solutions or plasma (Buxbaum et al., 1982). In order to modulate the electrostatic and steric components, we treated VSV with neuraminidase.

In this paper, we show that desialylation of VSV enhances fusion with the plasma membrane and entry via the endocytic pathway of cultured cells. To examine the role of surface charge, we also studied fusion of VSV with liposomes of different composition (Yamada & Ohnishi, 1986). A detailed analysis of steps leading to low-pH triggered fusion allows us to determine which components of the overall fusion process are dominated by viral envelope sialic acid.

EXPERIMENTAL PROCEDURES

Materials. Octadecylrhodamine (R18) was obtained from Molecular Probes (Junction City, OR). Neuraminidase (*Clostridium perfringens*, type V) was from Sigma Chemical Co. (St. Louis, MO). Phospholipids were from Avanti Polar Lipids (Birmingham, AL). Tissue culture media were obtained from Gibco Labs (Grand Island, NY). Purified vesicular stomatitis virus (VSV, Indiana strain) was obtained from J. Brown (University of Virginia, Charlottesville, VA). The virus was grown on monolayer cultures of baby hamster kidney cells and purified by sucrose velocity and density gradients (Thomas et al., 1985).

Cell Cultures. Vero cells (a monkey kidney cell line) were grown to confluency in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were lifted

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[1] Abbreviations: VSV, vesicular stomatitis virus; R18, octadecylrhodamine; PC, phosphatidylcholine; PS, phosphatidylserine; fdq, fluorescence dequenching.

from the flasks by incubation at 37 °C with 1.5 mL of 0.5 mg/mL trypsin and 0.2 mg/mL EDTA in PBS. Cells were washed 3 times in a solution containing 145 mM NaCl and 10 mM Hepes, pH 7.4 (Hepes–NaCl).

Labeling of VSV with R18 and Treatment with Neuraminidase. VSV (1.0 mg) was incubated with R18 (16 μL, 1 mg/mL in ethanol) at room temperature for 15–20 min. For desialylation, the reaction mixture was incubated in 2 mL of PBS containing (40 units) neuraminidase at 37 °C for 30–40 min. Controls were treated in parallel. At the end of the incubation, buffer was added to bring the volume of the incubation mixture to 6.0 mL, and the mixture was carefully layered on 50% sucrose in a thick-walled polycarbonate tube (10-mL capacity). The samples were centrifuged at 50000*g* for 50 min at 4 °C (rotor type 70.1, Beckman Model L8-M). The supernatants were discarded; the virus layer on the sucrose cushion was resuspended and passed through a Sephadex G-25M column (Pharmacia PD10) (Clague et al., 1990). Virus was eluted in the void volume. The recovery of untreated virus and neuraminidase-treated virus was 65 and 75%, respectively. Removal of terminal sialic acid was assessed in a number of different ways: (1) chemical analysis of residual sialic acid in the virus pellets using the thiobarbituric acid assay (Warren, 1959); (2) release of radioactivity from [^{3}H]-glucosamine-labeled virus; no detectable radioactivity was found after neuraminidase treatment of VSV which was metabolically labeled with [^{3}H]leucine; (3) hemagglutination inhibition of chicken erythrocytes by the A/PR8 strain of influenza virus (Compans, 1974). Neuraminidase-treated virus did not show any hemagglutination inhibition activity whereas untreated VSV under similar conditions had 3840 hemagglutination inhibition units/mL. Forty units of neuraminidase per milligram of VSV was sufficient for maximal desialylation.

Binding to Cells. R18-labeled virus (50–100 μg of viral protein) was added to 2 × 10^7 Vero cells and incubated at 4 °C for 30–40 min to form Vero–VSV complexes. The cells were washed with Hepes–NaCl buffer, resuspended in a small volume, and kept on ice until further use. Quantitation of binding of VSV to Vero cells was done according to Puri et al. (1988). Fifty micrograms of virus was incubated with 2 × 10^7 cells in a volume of 0.4 mL in Hepes–NaCl buffer at 4 °C for 45 min. Unbound virus was separated through phthalate oil. Radioactivity in the pellet and supernatant was analyzed. Values are expressed as ±SD (n = 3 from a single experiment).

Fusion Assay. Fifty microliters of virus–cell complexes was added to a cuvette containing 2.0 mL (pH 7.4) of buffer prewarmed at a given temperature. The mixture was stirred with a Teflon-coated 2 × 7 mm stirring bar. After 20–60 s, the fusion was triggered by addition of 10–100 μL of 0.5 M MES. The pH was always measured at the end of the run. Fluorescence changes were monitored using an SLM 8000 spectrofluorometer with 1-s time resolution at 560- and 590-nm excitation and emission wavelengths, respectively. A 570-nm cutoff filter was used at the emission to reduce scatter contributions. In some experiments, the fluorescence increase was followed at preset pHs. At the end of the run, Triton X-100 was added to 0.05% final concentration to get a 100% dequenching value. The percentage fluorescence dequenching (fdq) was calculated according to Blumenthal et al. (1987):

$$\% \text{ fdq} = 100[(F - F_0)/(F_t - F_0)] \quad (1)$$

where F, F_0, and F_t are fluorescence values at a given time, at zero time, and after disruption of the VSV–cell complexes with Triton X-100, respectively.

Table I: Binding of VSV to Vero Cells[a]

virus preparation	% virus bound[b]
VSV	32.2 ● 0.5
asialo-VSV	44.4 ± 0.5
R18VSV	59.3 ± 2.4
asialo-R18VSV	53.9 ± 1.7

[a] Fifty micrograms of virus was incubated with 2 × 10^7 cells in a volume of 0.4 mL in Hepes–NaCl buffer at 4 °C for 45 min. Unbound virus was separated through phthalate oil. Radioactivity in the pellet and supernatant was analyzed. [b] Values are expressed as ±SD (n = 3 from a single experiment).

Virus–Liposome Interaction. Large unilamellar liposomes were prepared by high-pressure extrusion through polycarbonate filters with 0.8-μm pore size (Nucleopore Corp., Pleasanton, CA) according to Hope et al. (1985). R18VSV (80 μg in 0.2 mL) was added to 0.5 mL of liposomes (1 mg of phospholipid) at pH 7.4, and the mixture was incubated at 4 °C for 30 min to allow association of virus and liposomes. The temperature was brought up to 37 °C, and samples were further incubated at pH 7.4 or 5.8 for 15 min. An aliquot of the incubation mixture was assayed for fluorescence before and after addition of Triton X-100 as mentioned above. Percentage dequenching was calculated according to eq 1 except that F_0 values were taken as fluorescence dequenching at pH 7.4, 37 °C for 15 min.

RESULTS

Binding to the Cells. Table I shows that labeling of VSV with R18 enhanced binding to Vero cells. However, no difference in binding was observed between R18VSV and neuraminidase-treated R18VSV. VSV binding to cell membranes appears to be predominantly electrostatic, since it is enhanced by labeling with positively-charged R18 (Blumenthal et al., 1987) or in the presence of positively charged DEAE-dextran (Bailey et al., 1984). Labeling with R18 appears to override the differences in charge between VSV and asialo-VSV. These results also demonstrate that sialic acid on the viral surface is not required for binding to Vero cells.

Kinetics of Fusion of VSV and Asialo-VSV with Cells. Figure 1A shows the time course of fusion of untreated and neuraminidase-treated R18VSV with Vero cells at pH 5.9. Fusion of untreated virus was about 40% in 400 s, whereas fusion of neuraminidase-treated virus was about 70% in 400 s. To assess the specificity of fluorescence dequenching, we pretreated virus preparations at 56 °C before binding to cells. Heat treatment of virus for 30 min inhibited fluorescence dequenching of both untreated and neuramindase-treated virus. These results clearly show that the enhancement in fusion after desialylation of R18VSV was not due to the artifactual dequenching but was the result of fusion of the viral membrane with the cell membrane. The extent of fusion mediated by VSV was enhanced after desialylation over the entire pH range (data not shown).

Figure 1B shows the increase in fluorescence upon fusion at pH 7.4, 37 °C. We have previously shown that fdq in pH 7.4 presents fusion in the endosome. As can be seen in Figure 1B, fdq of VSV at pH 7.4 was enhanced after desialylation. Fusion via the endocytic pathway was inhibited in the presence of 50 mM NH_4Cl, as expected for viral fusion events in endocytic vesicles (Blumenthal et al., 1987).

Role of Surface Charge on the Target Membrane. To test whether the reduced charge repulsion between the membranes

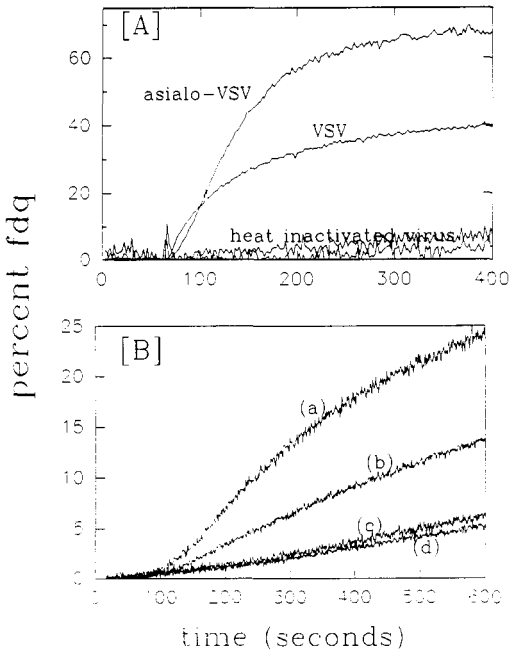

FIGURE 1: Kinetics of fusion of VSV and asialo-VSV with Vero cells. (A) R18-Labeled VSV or asialo-VSV were bound to Vero cells, washed, and suspended in NaCl–Hepes. Fifty microliters of the complex was injected into 2 mL of buffer at pH 7.4 and 37 °C. About 1 min later, the pH in the medium was lowered to 5.9 by adding 50 μL of 1 M MES. Percent fdq was calculated according to eq 1. To assess nonspecific dequenching, the virus suspension was incubated at 56 °C for 30 min and bound to cells, and fluorescence dequenching was followed at pH 5.9 as above. (B) fdq was followed at 37 °C and pH 7.4 in the absence (a and b, asialo-VSV and VSV, respectively) and presence of ammonium chloride (50 mM final concentration) (c and d, asialo-VSV and VSV, respectively) to assess entry via the endocytic pathway.

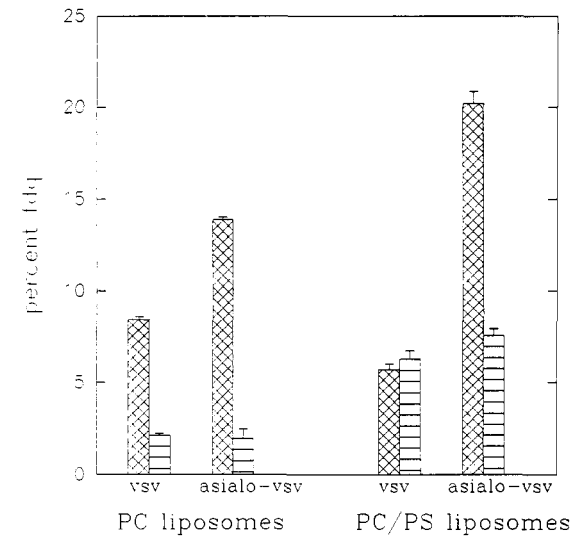

FIGURE 2: Fusion of R18-labeled VSV with liposomes. PC or PC/PS (1:1 mol/mol) liposomes were incubated with R18-labeled virus at pH 5.8 and 7.4 for 15 min and 37 °C as described under Experimental Procedures. Percent fdq was calculated according to eq 1 except that F_0 values were dq at pH 7.4, 37 °C, 15 min. The values are the mean of three determinations from a single experiment. Crossed-hatched bars, untreated VSV or asialo-VSV; horizontal striped bars, 56 °C treated virus.

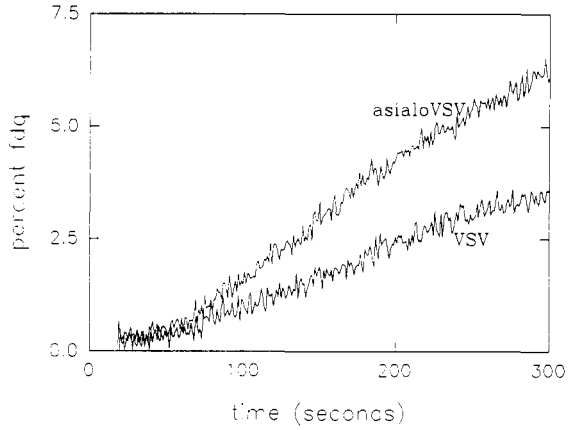

FIGURE 3: Lag times of fusion of VSV with Vero cells at 32 °C, pH 6.2. R18-Labeled virus was bound to Vero cells as described under Experimental Procedures. The virus–cell complexes were added at 20 s in 2.0 mL of preequilibrated Hepes–NaCl buffer at pH 6.2 and 32 °C, and fdq was monitored for 300 s. The curves shown in the figure represent the average of three kinetic runs.

resulted in enhanced fusion of asialo-VSV, we studied fusion of VSV with zwitterionic phosphatidylcholine (PC) and negatively charged phosphatidylserine (PS) (PS/PC, 1:1) liposomes. To observe the fusion, the virus needed to be incubated with liposomes at high concentrations, since fusion of VSV with those liposomes is quite inefficient. Under those conditions, we were not able to continuously monitor VSV–liposome fusion. Figure 2 shows the percent fdq upon low-pH incubation of VSV and asialo-VSV with liposomes, before and after treatment of virus at 56 °C to control for nonspecific fluorescence changes. Both VSV and asialo-VSV fused with PC liposomes as indicated by the fact that fluorescence dequenching was significantly above the background of fdq observed with heat-treated virus. As expected from studies with cells, asialo-VSV was more fusogenic. When VSV was incubated with PS/PC liposomes, no fdq above background was observed, indicating that those liposomes did not fuse with VSV. However, after desialylation, VSV did fuse with PS/PC vesicles (Figure 2).

Lag Times of Fusion. It has been previously shown using stopped-flow measurements that fusion of R18VSV with lipid-symmetric human erythrocyte ghosts is preceded by a lag phase (Clague et al., 1990). This is consistent with the notion that after triggering the viral envelope proteins undergo a series of activation steps before the final fusion event takes place. To examine the effect of desialylation on those activation steps, we compared the lag times of fusion of asialo-VSV and untreated VSV. Since the lag times reflect the rates of fusion, which is dependent on temperature and pH, we compared the lag times of fusion of untreated and asialo-VSV at 32 °C and pH 6.2. Under these conditions, we were able to observe lag times before fusion took place. The results are shown in Figure 3. We found that the lag times were the same for untreated and asialo-VSV (25 s), suggesting that the enhanced fusion activity of asialo-VSV is not due to faster activation reactions. This observation is consistent with the similar initial rates of fusion of VSV and asialo-VSV (see Figure 1A).

pH-Induced Inactivation of VSV Bound to Cells. Previously we have identified inactivation reactions of VSV bound to cells (Clague et al., 1990). Those reactions are apparent at pH values close to threshold where the activation reactions are slow. Figure 4 shows that fusion at pH 5.8 was considerably reduced when VSV–cell complexes were preincubated at 25 °C and pH 6.5, whereas little reduction was seen with asialo-VSV. Incubation of virus–cell complexes at pH 7.4 and 25 °C before lowering the pH did not result in a significant reduction of fusion activity of untreated virus and asialo-VSV (curves e in Figure 4). This indicates that the pH-induced decrease in fusion activity of prebound VSV was not due to dissociation of the virus from the cell surface.

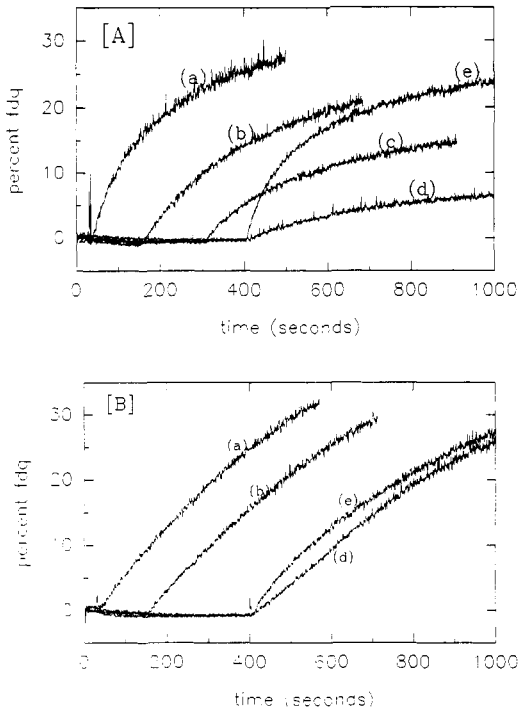

FIGURE 4: Effect of preincubation at pH 6.5 of VSV and asialo-VSV bound to Vero cells on low-pH-induced fusion. Fluorescently labeled VSV (A) or asialo-VSV (B) was bound to Vero cells as described under Experimental Procedures, and the samples were injected into Hepes–NaCl buffer at pH 7.4 and 25 °C. After 30 s, 0.5 M MES was added to adjust the pH to 5.85 (curve a) or 6.5 (curves b–d). The samples adjusted to pH 6.5 were incubated for different amounts of time after which the pH was brought down to 5.85 by further addition of MES. Curves e represent incubation of samples at pH 7.4 for 400 s before addition of MES.

DISCUSSION

Entry of VSV into Cells. Delivery of the viral nucleocapsid into cells is preceded by a complex set of biochemical and cell biological events which involve binding to the cell surface, endocytosis, and fusion between viral and cellular membranes. We have focused on factors that modulate the fusion event. Since electrostatic and steric constraints are important barriers to membrane fusion (Blumenthal, 1987), we chose to modulate the viral fusion process by interfering with those barriers. This is achieved by removing sialic acid moieties from the virual surface. Although such treatment did not affect the binding step, fusion activity at the plasma membrane as well as via the endosomal route was considerably enhanced (see Figure 1). Since it has previously been shown that neuraminidase treatment of VSV does not affect viral infectivity (Cartwright & Brown, 1977), we may surmise that enhanced activity of the viral envelope does not necessarily lead to increased infectivity.

Role of Surface Charge. Since desialylation is likely to reduce electrostatic repulsion between apposing membranes, we compared fusion of untreated VSV and asialo-VSV with uncharged and negatively charged liposomes (Figure 2). We found that incubation of R18-labeled virus with liposomes led to marked nonspecific fluorescence dequenching. In order to assess the amount of fusion, we subtracted the fluorescence dequenching resulting from incubation of liposomes with R18-labeled virus pretreated at 56 °C. Using that method, we found that VSV fused with uncharged PC liposomes and that desialylation enhanced its fusogenic activity with those liposomes. Surprisingly, VSV did not fuse with negatively charged liposomes. Fusion activity with those liposomes was only observed after deisalylation of VSV. Yamada and Ohnishi had reported that VSV fused equally well with liposomes composed of PC as well as PS/PC, as long as those phospholipids contained cis-unsaturated fatty acyl chains (Yamada & Ohnishi, 1986). Without performing the control for nonspecific lipid exchange, we would have arrived at the same conclusion.

It is also of interest to relate these findings to results with biological membranes. Although little PS may be present on the outer surface of nucleated cells (Rothman & Lenard, 1977), it is likely that VSV will find a phospholipid domain in biological membranes (Yamada & Ohnishi, 1986) devoid of the negatively charged lipid. Consistent with this hypothesis is our finding that VSV fused equally well with lipid-symmetric human erythrocyte ghosts before and after treatment with the enzyme PS-decarboxylase, which removed PS from the outer surface of the erythrocyte ghosts (Herrmann et al., 1990).

Dissection of Steps Leading to Viral Fusion. Studies of VSV fusion using rapid kinetic techniques indicate that the fusion does not follow a simple exponential behavior as would be expected from an instantaneous establishment of lipid continuity (Clague et al., 1990). The kinetics show a rather complex structure reminiscent of ion gating kinetics. At pH values close to threshold, a lag time of about 2 s was followed by a relatively slow rise. The lag time decreased and the rise time increased as the pH came closer to optimum. We interpret the lag time as reflecting the relative rates of rearrangements into the fusogenic state where membrane mixing takes place.

The final extents of fusion are also pH-dependent. A scheme with pH-dependent rate constants for the various activation steps would lead to the same extent of fusion irrespective of pH. To account for the pH dependence of the extent of fusion, we invoke an "inactivation" pathway. According to that model, pH-dependent viral fusion occurs when the envelope proteins undergo pH-activated conformational changes while the virus is appropriate bound to the target membrane. pH-dependent conformational transitions occurring while the virus is not in the proper apposition with respect to the target membrane will result in a failure to fuse, or "inactivation". If the rates of activation reactions leading to fusion are slow relative to those of the inactivation pathway, suboptimal extents will be reached, whereas rapid activation rates will yield optimal extents. Although the extent of fusion was enhanced 2-fold after desialylation (Figure 1), there was no change in the kinetics (i.e., lag times, see Figure 3). From those data, we inferred that the enhanced extent of fusion mediated by asialo-VSV is due to decreased inactivation. This hypothesis was confirmed by showing that desialylation caused a signifcant decrease in the rate of inactivation by exposure at pH 6.5 and 25 °C (Figure 4).

Model for Activation and Inactivation. A model which relates presumed conformational transitions of the VSV G-protein to mechanisms of viral fusion is shown in Figure 5. According to the model, VSV G undergoes a proton-driven shift from a "T" (tense) state at neutral pH either to the "R" (relaxed) state, which is fusion-active (Blumenthal, 1988), or to the "D" state, which is inactive (Clague et al., 1990). Kinetic data indicate that R and D represent a series of states (Clague et al., 1990), but for simplicity they are represented as one state. We hypothesize that the conformational changes result in movement of the fusion-active region of the protein either into the target membrane (R state) or not into the membrane (D state). In the case of influenza hemagglutinin, it has been shown that lowering the pH leads to movement of the fusion

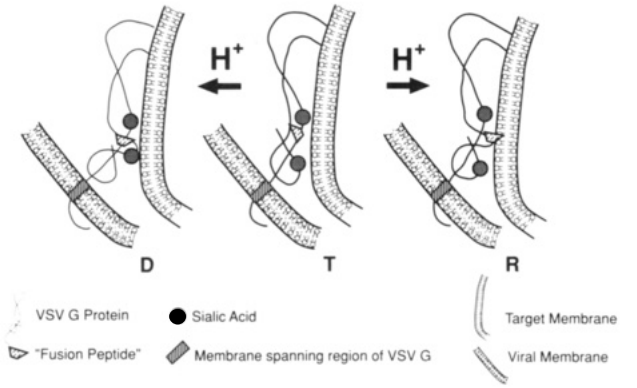

FIGURE 5: Model for VSV G-protein-mediated fusion and inactivation. Only one monomer of a presumed oligomeric complex is shown. The N-linked oligosaccharides which terminate in sialic acid are at amino acid residues 179 and 336 (Rose & Gallione, 1981). The fusion peptide is presumed to be an internal sequence of amino acid residues 118–136 (Ohnishi, 1988; Whitt et al., 1990). The low-pH-triggered conformational change is represented by a reorientation of the fusion peptide from inward (T state) to outward (R and D states). In the inactive D state, the fusion peptide is reoriented without inserting into the target membrane. Insertion into the target membrane (R state) is a step that leads to fusion. Only a small portion of the virual and target membranes is shown. See the text for further explanation.

peptide out of the trimer interface (White & Wilson, 1987). Moreover, on the basis of photolabeling studies, an activated state has been identified prior to fusion which involves insertion of some portion of the viral protein into the target bilayer (Stegmann et al., 1991). A dissection of steps leading to viral envelope protein-mediated membrane fusion indicates that formation of this kind of prefusion state is relatively rapid (Blumenthal et al., 1991). The observed delay times in viral fusion represent a series of subsequent events leading to the opening of a "fusion pore", which allows redistribution of lipids (Sarkar et al., 1989; Spruce et al., 1989).

For clarity, we present in Figure 5 only one monomer of the viral envelope protein which is presumably arranged in an oligomeric complex (Blumenthal, 1988; Doms et al., 1987; Crise et al., 1989; Zagouras et al., 1991). Moreover, in this scheme, we do not make an assumption as to the orientation of the protein, which has been hypothesized to be oriented perpendicular (Bentz et al., 1991), parallel (Stegmann et al., 1991), or at a 55° angle (Guy et al., 1992) with respect to the viral bilayer. Although VSV G does not have a cleaved N-terminal fusion peptide, an internal sequence of 19 uncharged amino acids (residues 118–136) has been proposed as a possible "fusion peptide" (Ohnishi, 1988; Whitt et al., 1990). How might sialic residues on the G-protein affect insertion of the fusion peptide into the bilayer (R state) or exposure without insertion (D state)?

VSV G contains two N-linked oligosaccharides at amino acids 179 and 336 (Rose & Gallione, 1981). We propose that one or both are in the neighborhood of the "fusion peptide". Acid-activated exposure of the fusion peptide in the vicinity of negatively charged sialic acid residues might prevent its insertion into bilayers with negatively charged headgroups. This notion is consistent with results presented in Figure 2 which indicate that PC liposomes are susceptible to VSV fusion whereas PS/PC liposomes are not. Since desialylation removes the negatively charged obstacles on the G-protein, the PS/PC liposomes become susceptible to fusion with asialo-VSV (see Figure 2).

The effects of desialylation of VSV on the rates of activation and inactivation and the extents of fusion with biological membranes can also be explained on the basis of this model. Since formation of the R state is not the rate-limiting step in the overall process of fusion, the rates and delays of fusion are the same for VSV and asialo-VSV (see Figures 1 and 3). We interpret inactivation (transition into the D state) as exposure of the fusion peptide without inserting into the target membrane (see Figure 5). The presence of sialic acid residues in the vicinity of the fusion peptide will obstruct insertion, and therefore enhance transition into the D state. This notion is consistent with the observation that inactivation was significantly reduced following desialylation of VSV (see Figure 4). The overall extents of fusion are derived from a balance between the two competing pathways. A reduction of the rate of transitions into the D state by desialylation will therefore lead to an enhanced extent of fusion as shown in Figure 1. We plan to test the model further by examining the fusogenic activity of mutants of the G-protein with altered glycosylation sites (Machamer & Rose, 1988; Pitta et al., 1989).

In conclusion, we have dissected steps in VSV G-protein-mediated fusion by studying the kinetics of viral fusion before and after desialylation. To analyze the fusion kinetics of the two forms of VSV, we have developed a working hypothesis, which forms the basis for further experimentation. The combination of these biophysical techniques with molecular biological approaches will yield further insights into the intricacies of the viral envelope protein-mediated membrane fusion.

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