Low-pH Conformational Changes of Rabies Virus Glycoprotein and Their Role in Membrane Fusion

YVES GAUDIN,^{1*} ROB W. H. RUIGROK,² MARCEL KNOSSOW,³ AND ANNE FLAMAND¹

Laboratoire de Génétique des Virus, Centre National de la Recherche Scientifique, 91198 Gif sur Yvette cédex,¹ EMBL Grenoble Outstation c/o ILL, 38042 Grenoble cédex,² and Laboratoire de biologie structurale (CNRS UMR 9920), Université de Paris Sud, 91405 Orsay cédex,³ France

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Fusion of rabies virus with membranes occurs at acidic pH and is mediated by the viral spike glycoprotein (G). In this paper, we provide the basis for a description of structural transitions associated with exposure to low pH and of their role in membrane fusion. First, we have extended previous studies of fusion kinetics and we have shown that low-pH inhibition of fusion is detectable at 0.5 pH units higher than fusion. Second, low-pH-induced conformational changes were analyzed by using electron microscopy and monoclonal antibody binding assays. The existence of a pH-dependent equilibrium between the native and a low-pH inactive conformation was demonstrated. Third, besides these two conformations, we, using the fluorescent probe ANS (8-anilino-1-naphthalenesulfonic acid), provide evidence for the existence of a transient third state which appears to be more hydrophobic than the native state. Our results suggest that this transient state is responsible for viral aggregation at low pH and could play a role in the first steps of the fusion mechanism.

Many enveloped viruses enter cells by a process of receptor-mediated endocytosis (9). Subsequently, the viral envelope fuses with the endosomal membrane to allow the release of the viral genome into the cytoplasm. This step is induced by the low pH of the endocytic compartments and is mediated by a viral glycoprotein; the best-characterized fusogenic glycoprotein is influenza virus hemagglutinin (HA) (17, 20, 23).

Rabies virus, a rhabdovirus, causes a fatal disease associated with intense replication in the central nervous system. Its unique glycoprotein (G) is a homotrimeric integral membrane protein of approximately 200 kDa that forms a spike extending 83 Å (1 Å = 0.1 nm) from the viral membrane (5). This glycoprotein initiates the interaction between the virus and the target cell: it is responsible for virus attachment to specific receptors (24). It also bears the fusion properties of the virus (22) which are detectable below pH 6.2 (6, 10, 22).

The mechanism by which the rabies virus glycoprotein catalyzes membrane fusion is far from being understood. We have previously shown that, at an acidic pH, G undergoes conformational changes and that a preincubation of the virus at an acidic pH in the absence of membranes leads to inhibition of its fusion properties (6). These features are common to rabies and influenza viruses (15, 21). The main difference is that, in the case of rabies virus, both the loss of fusion properties and conformational changes appeared to be reversible by readjusting the pH to neutral (6).

In this work, we show that for the CVS strain of rabies virus, fusion inhibition is detectable at 0.5 pH units higher than fusion. By using electron microscopy (EM) and monoclonal antibody (MAb) binding assays, we identify and characterize the associated conformational change. Furthermore, we demonstrate the existence of a pH-dependent equilibrium between the native and the inactive conformation and show that when the equilibrium reaches about 50% native-50% inactivated G, the viral fusion properties are lost. Besides these two conformations, we provide evidence for a

transient exposure of hydrophobic regions that are buried in the native conformation. Our observations suggest that this transient state is involved in the first steps of the fusion mechanism. Taken together, these results provide the basis for a description of structural transitions associated with exposure to low pH and their relationships with the fusion phenomenon.

MATERIALS AND METHODS

Chemicals. *N*-(Lissamine rhodamine B sulfonyl)-phosphatidylethanolamine (RHO-PE) and *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)-phosphatidylethanolamine (NBD-PE) were purchased from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Phosphatidylcholine, phosphatidylethanolamine, and gangliosides (type III from bovine brain) were supplied by Sigma Chemical Co.

Viruses and MAbs. The CVS strain of rabies virus and the antigenic avirulent mutant 50L1, mutated at amino acid 333 of the viral glycoprotein (Arg to Glu), were cultivated on BSR cells and purified as previously described (11, 14).

Seven neutralizing anti-G MAbs with different specificities were used. They were produced in BALB/c mice immunized with UV-inactivated strain CVS virus and characterized in our laboratory (1, 11, 14).

Buffers and pH adjustment. The buffer we used to adjust the pH was phosphate-citrate buffer prepared from a 0.1 M solution of citric acid and 0.2 M dibasic sodium phosphate stock solutions. Viral dilutions in these buffers do not modify the pH. Readjustment to slightly basic pH was made by adding the appropriate volume of the 0.2 M phosphate solution.

Preparation of liposomes and assay for fusion. A total of 250 µg of phosphatidylcholine, 500 µg of phosphatidylethanolamine, and 250 µg of gangliosides dissolved in organic solvents were mixed with 10 µg of RHO-PE and 10 µg of NBD-PE and dried in vacuo. After addition of 1 ml of 0.15 M NaCl, the mixture was sonicated for six 3-min periods. The liposome suspension was clarified at 3,500 × *g* for 5 min, and the supernatant was used in the subsequent assay.

* Corresponding author.

Fusion was assayed as previously described (6) by using the resonance energy transfer method of Struck et al. (19). Briefly, 10 μ l of fluorescent liposomes was mixed with 980 μ l of phosphate-citrate buffer at the required pH, and then 10 μ l of virus was added and the increase of NBD fluorescence was monitored continuously. Fluorescence was measured with a thermostated Jobin Yvon JY3 spectrofluorimeter with excitation and emission slit widths set at 10 nm. Excitation was set at 455 nm, and emission was set at 535 nm. The mixture was kept under continuous stirring during the experiment.

EM. Samples were diluted with phosphate-buffered saline (PBS) (150 mM NaCl and 10 mM phosphate buffer, pH 7.4) or phosphate-citrate buffer at the required pH to a viral concentration of 1 mg/ml, adsorbed on carbon film, and then negatively stained with 1% sodium silicotungstate (pH 7.1) as previously described (5, 12). EM experiments were performed on the CVS strain, on avirulent mutant 50L1, and on both types of viruses inactivated with betapropiolactone. We did not notice any difference between these different samples.

Indirect ELISA. Virions were first incubated at 37°C in phosphate-citrate buffer at the required pH for various periods of time. Then each sample was twofold serially diluted in phosphate-citrate buffer, pH 6.4, at 18°C. (It was impossible to dilute the virus at neutral pH because such a reincubation would have immediately led to recovery of native antigenicity. These conditions of dilution were chosen because the kinetics of G conformational changes were significantly slowed down at pH 6.4 and 18°C [in this case the equilibrium was not reached after 5 h of incubation].) Each viral dilution was mixed with ascitic fluid previously diluted in phosphate-citrate buffer, pH 6.4. The ascitic fluid dilution had been chosen in the range in which the enzymatic response was linear in an enzyme-linked immunosorbent assay (ELISA). After 1 h of incubation at 18°C, free antibodies were titrated by an ELISA performed as described elsewhere (11).

Interaction of rosettes with ANS. Glycoprotein solubilized and purified as previously described (5) was extensively dialyzed against PBS (150 mM NaCl, 10 mM phosphate, pH 7.4) containing Bio-beads SM2 (Bio-Rad) to eliminate the detergent.

ANS (8-anilino-1-naphthalenesulfonic acid) fluorescence was measured with the same spectrofluorimeter as for fusion (excitation and emission slit widths were set at 10 nm). Excitation was set at 360 nm, and the emission spectrum was recorded between 410 and 560 nm. The ANS concentration was 25 μ M, and the protein concentration was 40 μ g/ml. Rosettes and ANS were kept in the dark for 1 min under constant stirring to allow interaction before recording the spectrum. The temperature was kept at 23°C during the experiment.

RESULTS

Characteristics of fusion induced by rabies virus. The resonance energy transfer method of Struck et al. (19), which involves the nonexchangeable probes NBD-PE as a fluorescent donor and RHO-PE as a fluorescent acceptor, was used to assay for fusion. Fusion of fluorescent liposomes with viral membranes results in dilution of the fluorescent probes and a consequent increase in fluorescence emission of NBD-PE.

Figure 1 shows typical kinetics of fusion obtained under different pH and temperature conditions. As previously

FIG. 1. Fusion of rabies virus, strain CVS, with liposomes at pH 5.28 and 37°C (A), pH 5.90 and 18°C (B), pH 5.92 and 37°C (C), and pH 6.12 and 18°C (D) (see Materials and Methods). Fluo., fluorescence.

described (6), fusion was preceded by a short lag time. The duration of the lag increased with the pH and decreased at high temperatures (Fig. 2B). We did not observe any significant difference in the pH threshold of fusion at 37 or 18°C; however, the extent of fusion was less at 37°C (Fig. 2A). At both temperatures, fusion was detectable only at or below pH 6.2. The fusion extent was maximal around pH 5.9 at 18°C and around pH 5.5 at 37°C, and then it decreased with the pH. The rate of fusion was also dependent on pH and temperature (Fig. 2C). Just below the pH threshold of fusion, the rate was higher at 18 than at 37°C. At pH 5.6 and below, the converse was true.

Effect of viral preincubation at low pH. We have previously shown that a preincubation of the virus at pH 5.9 in the absence of a target membrane inhibited fusion (6). Here, we studied this inhibition more thoroughly by examining its dependence on pH and temperature (Fig. 3). This phenomenon was more rapid when the temperature was high and the pH was low. Preincubation of the virus at pH 6.7 (0.5 pH units above the pH threshold for fusion; see above) and 37°C for 2 h led to a complete inhibition of the viral fusion properties, whereas a preincubation of the virus at pH 6.8 and 37°C for 2 h had no significant influence on the viral fusion properties.

In contrast to influenza virus, the low-pH inhibition of the fusion properties of rabies virus is reversible by readjusting the pH to neutral (6). We have studied the kinetics of the recovery of the fusogenic capacity of the virus. When virions had been incubated for 2 h at 37°C and pH 6.4 or 6.7 (and consequently had lost their ability to fuse liposomes), they completely recovered their initial fusion capacities after a 40-s reincubation at pH 7.3 and 25°C, indicating that the inhibition was totally reversible (data not shown).

Initial experiments indicated that G undergoes reversible conformational changes at low pH (6). The exact nature of these structural transitions and their role in the process of fusion remained to be determined. Therefore, in the following section we, using EM and MAb binding assays, will describe the kinetics and the pH dependence of these structural changes.

EM. We used EM of rabies virus suspensions to study the viral aggregation and the modification of the morphology of

FIG. 2. Characteristics of fusion induced by rabies virus. (A) pH dependence of the extent of fusion at 18 and 37°C. Note that the ordinate scale is different at 18 and 37°C to clearly show that the pH threshold is the same at both temperatures. (B) pH dependence of the duration of the lag phase (at 18 and 37°C). Using our technique, we could not measure lag time shorter than 6 s. The lag time at pHs above 6.15 was difficult to measure because of the very low initial rate of fusion. (C) pH dependence of the rate of fusion (at 18 and 37°C). The rate was calculated from maximal slopes of kinetic curves shown in Fig. 1. Fluoro., fluorescence.

the surface glycoproteins at low pH. We performed both negative-staining EM and cryo-EM of samples in a frozen hydrated state (data not shown). We obtained identical results with both methods, indicating that the morphology of low-pH G was not influenced by the negative-staining procedure (we used sodium silicotungstate at pH 7.1 as the stain).

As previously described (6), we detected massive aggregation of the virus at low pH. This phenomenon was pH and temperature dependent: at pH 6.4 and 6.7, aggregation was detectable at 0 but not at 37°C (all results and measurements obtained with EM are summarized in Table 1). At pH 5.9, aggregation was clearly detected at 37°C but was less extensive than at 0°C.

After an incubation at pH 5.9 and 18 or 37°C, the spikes visible at the edges of the aggregates had a different morphology from that observed at neutral pH: in contrast to the well-defined 83-Å-long native spike, low-pH spikes formed a rather wavy layer 113 Å wide (Table 1; Fig. 4a), reminding one of the difference seen between the native and low-pH spike layers of influenza virus (13). These lengthened spikes

FIG. 3. Kinetics of the inhibition of fusogenic properties of the virus at different pHs and temperatures. The virus was preincubated in a phosphate-citrate buffer at the required pH. Aliquots were taken at different time points and immediately tested for their ability to fuse liposomes.

were not observed on the edges of viral aggregates obtained at 0°C; under these conditions, the spikes conserved their native structure (Fig. 4b).

When virions were incubated at pH 6.7 and 37°C for 2 h, spikes of both lengths (83 and 115 Å) were present in the viral membrane (Fig. 4c). We estimated that roughly 50% of the viral spikes conserved their native length. This proportion decreased to 10% when the virus was incubated at pH 6.4 and 37°C for 2 h (Fig. 4d; Table 1). Furthermore, we found that after such preincubations (pH 6.4 or 6.7 at 37°C for 2 h) no aggregation occurred and that the pH could subsequently be lowered to 5.9 without leading to aggregation (Table 1); in this latter case, all the spikes appeared to be in the long conformation. These experiments clearly showed that the lengthened spikes were not responsible for viral aggregation. When all these viral preparations were subsequently incubated at pH 7.5, the spike morphology immediately reverted to the native one (Table 1; Fig. 4e).

Aggregation was also a reversible phenomenon: incubation of aggregates at pH 6.4 and 37°C led to total disaggregation and a low-pH spike morphology (Table 1). Incubation of aggregates at pH 7.5 immediately resulted in a native spike morphology (Table 1; Fig. 4e) but resulted in only very slow disaggregation (after a 10-min incubation at pH 5.9 and a subsequent overnight incubation at pH 7.5, many aggregates were still observed but were smaller in size).

We summarize these observations as follows. In all EM performed, we found only two spike morphologies, either the native 83-Å or the low-pH 115-Å spike, the percentage varying with the pH. The rate of the change from native to low-pH spike was considerably slowed down at low temperature: at pH 5.9 and 37°C, low-pH spikes were observed almost immediately at the outside of the aggregates, but at pH 5.9 and 0°C, we observed only a few lengthened spikes after 1 h of incubation.

Modification of the structure of antigenic sites of G at acidic pH. Major antigenic site II of G (defined in reference 1) has been shown to be modified at acidic pH (6). Here, using an

TABLE 1. EM observations and measurements^a

Expt no.	Virus treatment						Viral aggregation ^b	Spike morphology	Spike length ^c (Å)/no. of measurements
	First incubation			Second incubation					
	Time (min)	pH	Temp (°C)	Time (min)	pH	Temp (°C)			
1		7.3					—	Native	84 ± 5/80
2	5	6.4	0				+	Native	84 ± 4/56
3	5	6.4	0	90	6.4	37	—	90% changed	114 ± 12/77 ^d
4	120	6.4	37				—	90% changed	115 ± 8/68 ^d
5	120	6.4	37	5	7.5	18	—	Native	84 ± 5/26
6	120	6.4	37	10	5.9	18	—	Changed	116 ± 7/38
7	10	5.9	18				+	Changed	113 ± 13/36 ^e
8	10	5.9	18	90	6.4	37	Disaggregation	Changed	ND ^f
9	10	5.9	18	90	7.5	37	Slow disaggregation	Native	83 ± 4/23

^a The results obtained at pH 6.7 were the same as those at pH 6.4, but only 50% of the spikes were changed at the equilibrium at pH 6.7. Measurements for CVS and for the 50L1 mutant were identical.

^b +, aggregation; —, no aggregation.

^c Extending from the membrane.

^d Measurement of the lengthened spikes. The native spikes were not taken into account.

^e These measurements were also performed on virus in frozen hydrated suspension and gave values of 111 ± 12 Å for the spike length (30 measurements).

^f ND, not determined.

indirect ELISA, we have provided evidence that the kinetics and the pH dependence of the conformational change of site II are identical to the kinetics and the pH dependence of the conformational change detected by EM.

Figure 5A shows the kinetics of the conformational change at pH 6.4 and 37°C detected by MAb 30AA5 directed against antigenic site II. The experimental curves were shifted to the right when the duration of the preincubation increased, indicating that the epitope was modified. However, an equilibrium seemed to be reached after 1 h and 20 min, as the system did not evolve any further. Using the same MAb, we have also studied the extent of the conformational change at different pH values. Figure 5B shows the effect of viral preincubation for 2 h at 37°C and pH 7.3, 6.7, 6.4, or 5.9. The experimental curves were shifted to the right when the pH decreased, indicating that the extent of the conformational change detected by 30AA5 was more important at low pH. Assuming that 30AA5 exclusively recognized the native glycoprotein and consequently did not bind G in its low-pH conformation, we estimate that around 50% of the glycoprotein was in the low-pH conformation at the equilibrium at pH 6.7. This proportion reached 80% at pH 6.4 and exceeded 95% at pH 5.9. Experiments performed with two other MAbs directed against site II gave very similar results.

In this assay, too, the conformational change detected by 30AA5 appeared to be reversible. After a preincubation for 2 h at pH 6.4 and 37°C, 10 min of reincubation at pH 7.6 and 20°C was sufficient for the virus to recover its native antigenicity. The EM and MAb assays show that there exists a pH-dependent equilibrium between native G and low-pH G. The rate at which this equilibrium shifts after a change in pH depends on the temperature.

The kinetics of the low-pH conformational change detected in EM or with MAbs were similar to the kinetics of the low-pH inhibition of viral fusion. This indicates that G in its low-pH conformation was in an inactive state, unable to induce membrane fusion.

Evidence of the existence of a third conformation of G exposing hydrophobic regions at its surface. The EM experiments have shown that neither the native nor the lengthened spikes were responsible for virus aggregation (Table 1). Aggregation was probably due to a third state of G which exposed a previously buried hydrophobic region at its sur-

face. In order to identify species of G with increased hydrophobicity, we used the fluorescent probe ANS to monitor the hydrophobicity of rosettes of G as a function of pH. The ANS emission characteristics vary with the polarity of its environment: the quantum yield increases and the emission maximum shifts to the blue when the medium surrounding the probe is more hydrophobic. Rosettes of G were then mixed with ANS at different pHs, and the spectrum was recorded after 1 min of incubation (see Materials and Methods).

In the presence of rosettes, ANS fluorescence is stronger at pH 6.4 (Fig. 6, spectrum c) than at pH 7.3 (Fig. 6, spectrum b). The maximum of the spectrum is shifted from 502 (pH 7.3) to 489 (pH 6.4) nm. This increase of fluorescence and this blueshift of the emission maximum were also detectable at pH 6.7, although to a lesser extent (data not shown). The same experiments performed with the virus instead of rosettes gave similar results (data not shown). Therefore, lowering the pH below 6.7 results in the protein becoming more hydrophobic. This observation was made within 2 min of acidification at a time when no other modification (i.e., lengthening of the spike or alteration of its antigenicity) was detected.

To examine the effect of this transition on fusion, we preincubated the virus with liposomes at pH 6.4 and 0°C before inducing the fusion process at 18°C by lowering the pH to 6.18 (at pH 6.18 and 18°C, the lag is about 2 min). After preincubation at 0°C and pH 6.4, the lag decreased and the extent of fusion increased (Fig. 7). This effect on the fusion curves was still observed when the virus was preincubated with liposomes at pH 6.7 and 0°C (data not shown), whereas a preincubation at pH 7.3 and 0°C did not modify the experimental curves (data not shown). Taken together, these results indicate that the virus is able to interact with liposomes at slightly acidic pH (6.4 or 6.7) and 0°C in a manner which shortens the lag observed. They also suggest the establishment of a particular interaction between virions and liposomes at pH 6.4 or 6.7 and 0°C.

This lag shortening observed at 0°C between virions and liposomes at pH 6.4 and 6.7 appeared to be reversible: after a reincubation of the mixture at pH 7.5 for 10 min, the fusion curves recovered their initial aspect.

FIG. 4. Electron micrographs of low-pH-incubated rabies virus, negatively stained with sodium silicotungstate. All micrographs have the same magnification. Bar, 500 Å. (a) Rabies virus after 10 min of incubation at pH 5.9; (b) virus after 10 min of incubation at pH 6.4 and 0°C; (c) virus after 2 h of incubation at pH 6.7 and 37°C; (d) virus after 2 h of incubation at pH 6.4 and 37°C; (e) same virus as in panel a after subsequent incubation at pH 7.5.

FIG. 5. Conformational changes in antigenic site II. (A) Kinetics of the conformational changes at pH 6.4 and 37°C. The virus was incubated at pH 6.4 and 37°C for indicated times. Then it was twofold serially diluted in a buffer at pH 6.4 and 18°C, and subsequently antibodies (MAb 30AA5) were added. After a 1-h incubation, free antibodies were titrated in an ELISA test (see Results and Materials and Methods). (B) pH dependence of the extent of the conformational change. The virus was incubated at the indicated pHs for 2 h at 37°C and subsequently treated as described for panel A.

DISCUSSION

In this paper, we have reported the low-pH-induced conformational changes of rabies virus glycoprotein and their relation to the mechanism of fusion and fusion inhibition. First, we have extended previous studies of fusion kinetics, the most important result being that fusion inhibition is detectable at 0.5 pH units higher than fusion. This result contrasts with those obtained for influenza A virus HA, for which low-pH conformational changes, fusion inhibition, and fusion occur at the same pH (15, 21). Second, we have demonstrated the existence of a pH-dependent equilibrium between the native and an inactive state (I) of the glycoprotein. The inactive conformation is 32 Å longer than

FIG. 6. ANS fluorescence. The excitation wavelength was 360 nm, and the temperature was kept at 23°C. ANS (25 µM) was tested alone in PBS, pH 7.3 (a), or in the presence of 40 µg of G-rosettes per ml of PBS, pH 7.3 (b), or phosphate-citrate buffer, pH 6.4 (c). Fluorescence of ANS alone or in the presence of 0.02% CHAPS {3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate} does not vary in the pH range of our study (data not shown). A. U., arbitrary units.

the native one and is no longer recognized by MAbs directed against site II. Third, besides these two conformations, we have provided evidence for the existence of a more hydrophobic state that is otherwise (i.e., through morphology or MAb recognition) indistinguishable from the native state. This hydrophobic state is detectable at pH 6.7 and may play a role in the fusion mechanism. The transition to the hydrophobic state occurs immediately after acidification as shown with the fluorescent probe ANS. After this first structural

FIG. 7. Effect of a preincubation of the virus with liposomes at pH 6.4 and 0°C. For A, a total of 20 µl of virus was mixed with 970 µl of phosphate-citrate buffer (pH 6.18) containing 10 µl of fluorescent liposomes. Fusion was then monitored at 18°C as described in Materials and Methods. For B, a total of 20 µl of virus was mixed with 10 µl of liposomes and 20 µl of phosphate-citrate buffer (final pH, 6.4) and kept on ice for 5 min. The mixture was then added to 950 µl of phosphate-citrate buffer (pH 6.18), and the fusion was monitored at 18°C as previously described. Fluo., fluorescence.

FIG. 8. Conformational states of rabies G protein and their role in membrane fusion. N, native structure of G; A, activated conformation which exposes hydrophobic regions; I, inactive structure which appears lengthened in EM and which no longer interacts with MAbs directed against site II; X, association of an activated spike and the target membrane; X_n*, fusion complex. The subscript n indicates that probably more than one spike is involved in the fusion mechanism (see text).

transition, G is in an activated state and is able to interact with the target membrane in a manner different from that at neutral pH. This interaction seems to constitute a preliminary step in the fusion process, as a preincubation of the virus with liposomes at pH 6.4 or 6.7 at 0°C suppresses (or at least considerably reduces) the lag time. This latter point also shows that the first events preceding fusion can occur at pHs at which fusion is not observed. These events are reversible by readjusting the pH to neutral.

The onset of the activated state coincides with the viral aggregation and also with the optimum of the hemagglutinating properties of the virus (6–8). The fact that viral aggregation and hemagglutination are more easily detected at 0°C suggests that both phenomena involve hydrophobic interactions, which are known to be more stable at low temperatures. Similar observations have also been made for influenza A virions (strain X47), which at pH 5.1 and 0°C bind liposomes in a hydrophobic, salt-resistant manner (16, 18).

The subsequent conformational change detected by EM or MAb occurs more slowly and leads to the inactivation of the fusogenic properties of the glycoprotein. This inactivated state (I) is in a pH-dependent equilibrium with the native state. The pH at which 50% of the molecules are in an inactive state is about 6.7.

These considerations lead us to propose a model (Fig. 8) for the conformational changes of rabies virus glycoprotein and their role in membrane fusion. It is similar to the model proposed by Clague et al. (3) for vesicular stomatitis virus. Here, we have identified and characterized the activated and

inactivated states of the glycoprotein, the existence of which was only postulated on the basis of an analysis of the kinetics of fusion in the case of vesicular stomatitis virus (3).

It has been suggested that more than one trimer is required to induce fusion in the case of influenza A virus hemagglutinin and vesicular stomatitis virus glycoprotein (2, 4). Here, we show that, when only half of the spikes are in an inactivated state (after a 2-h viral preincubation at pH 6.7 and 37°C), rabies virions are unable to induce membrane fusion, and this also suggests the requirement for several trimers in the fusion complex in the case of rabies virus.

Finally, although at least some of the events observed during the lag can occur at pH 6.7, fusion is not detected above pH 6.2. This may signify that, after the initial activation of G, a second step of protonation is necessary for the activation of the fusion complex. Alternatively, we cannot exclude that, above pH 6.2, the surface density of glycoprotein in the activated (A) state is below the threshold required to induce fusion.

The model proposed here shares aspects with models proposed for influenza virus-induced membrane fusion (18), suggesting that the mechanisms by which these viruses fuse with target membranes may be similar. Particularly, as in the case of influenza virus HA, before being fusogenically active, the rabies virus glycoprotein is in an activated state which exposes hydrophobic regions at its surface and allows its interaction with the target membrane. The major difference between rhabdoviruses and influenza viruses is the reversibility which is observed in all structural transitions that occur at low pH and lead to fusion.

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