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Partial Fusion Activity of Influenza Virus toward Liposomes and Erythrocyte Ghosts Is Distinct from Viral Inactivation*

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Final extents of fusion of influenza virus (A/PR/8/34 strain) with neutral and partially acidic liposomes were monitored with (i) a fluorescence resonance energy-transfer assay in which the liposomes were labeled and (ii) by the dequenching of octadecylrhodamine, initially incorporated in the viral membrane. The latter assay was also employed in the fusion of influenza virus and Sendai virus with erythrocyte ghosts. In all cases, a phenomenon of partial fusion activity of the virus was observed, which is distinct from low pH inactivation. The unfused influenza or Sendai virions, which were separated by sucrose gradient centrifugation from liposomes or erythrocyte ghosts exhibited again partial fusion activity toward freshly added liposomes or ghosts, respectively. The conclusion is that the fraction of initially bound and unfused virions does not consist of defective particles, but rather of particles bound to the target membranes via inactive sites on the virus (or on cellular membranes), or else, partial fusion activity is a manifestation of a certain probability of production of fusion inactive sites by irreversible association of viral glycoproteins or peptides in the target membrane.

Analysis of final extents of virus-liposome fusion demonstrates that all three viruses studied, influenza virus (1–4), Sendai virus (5–7), and HIV-1[1] (8), exhibit partial fusion activity toward liposomes of certain compositions, *e.g.* in certain cases only 20–40% of the virions fuse. For influenza virus and Sendai virus fusing with cells, the results in several cases indicate complete fusion activity, *i.e.* close to 100% of the virus particles can fuse with erythrocyte ghosts and with several suspension cells, provided that the number of virions per cell does not exceed a certain limit of the order of tens to hundreds, depending on the size and composition of the cellular membranes (3, 9–14). However, other studies (15) do indicate incomplete fusion activity of these viruses with cellular membranes. Furthermore, in the case of several immunodeficiency viruses, such as HIV-1, only a small fraction of the virions fuse with the cellular target membranes (8, 16).

In the current study, we first present details of the mechanisms of partial fusion activity of influenza virus toward liposomes. At pH 5 and 37 °C, influenza virus exhibits 100% fusion activity toward the acidic liposomes PS and cardiolipin, whereas only 20–40% of the virions are capable of fusing with liposomes composed of PC/PE with or without cholesterol and gangliosides (2, 4). The phenomenon of partial fusion activity should not be confused with low pH inactivation of fusion activity that has been observed in the case of influenza virus (3, 4, 13–15, 17–21). Thus, the reduction in the fusion rate constant upon exposure of influenza virus to low pH is similar when the target membrane of a liposome is composed of PS or PC/PE/cholesterol with or without gangliosides, but the final extents of fusion are 100% and 20–40%, respectively (4). Furthermore, at neutral pH, Sendai virus and HIV-1 are not inactivated but they exhibit partial fusion activity toward liposomes. Following a previously described procedure (22), results with Sendai virus fusing with liposomes[2] (3), indicated that bound, unfused virions can be released by sucrose gradient centrifugation. The separated unfused virions subsequently fused when incubated with a "fresh" batch of liposomes. Hence, the fraction of initially bound unfused Sendai virions does not consist of defective particles, but rather of particles bound to liposomes via "inactive" sites.

In the current study, we also looked at the effect of temperature on the final extents of fusion of Sendai virus and influenza virus with liposomes and erythrocyte ghosts. Interestingly, in most cases, the fraction of fusing virions increased with temperature, despite the accelerated inactivation of influenza virus at higher temperatures.

EXPERIMENTAL PROCEDURES

Materials

PC, PS, and PE were purchased from Avanti Polar Lipids Inc. (Birmingham, AL). Cholesterol and gangliosides were from Sigma, $C_{12}E_8$ was from Calbiochem, R18, NBD-PE and Rh-PE were from Molecular Probes Inc. (Eugene, OR).

Methods

Virus Preparations—Influenza virus, A/PR/8/34 (H1N1) strain, was obtained from SPAFAS Inc. (Preston, CT). The virus was grown for 48 h at 37 °C in the allantoic cavity of 11-day-old embryonated eggs, purified by discontinuous sucrose density gradient centrifugation and stored at −70 °C in phosphate-buffered saline. Sendai virus (hemagglutinating virus of Japan) Z strain was obtained from SPAFAS Inc. (Preston, CT). The virus was grown for 72 h in the allantoic cavity of 10-day-old embryonated eggs, purified by differential centrifugation, and stored at −70 °C in phosphate-buffered saline.

Liposome Preparation—Large unilamellar lipid vesicles (LUVs) were prepared by the reverse phase evaporation method in 150 mM NaCl, 10 mM Hepes, pH 7.4, as described (23). The vesicles were sized through 0.1-μm polycarbonate filters, and their concentration was determined

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[1] The abbreviations used are: HIV, human immunodeficiency virus; $C_{12}E_8$, octaethylene glycol dodecyl ether; LUVs, large unilamellar vesicles; NBD-PE, *N*-(-7-nitro-2,1,3-benzoxadiazol-4-yl) phosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; R18, octadecylrhodamine B chloride; RET, resonance energy transfer; Rh-PE, *N*-(lisamine rhodamine B sulfonyl) phosphatidylethanolamine; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

[2] S. Nir, K. Klappe, H. Hoff, and D. Hoekstra, unpublished data.

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TABLE I
Final intensity (I) levels and percents of virions fusing for influenza virus labeled by R18 interacting with excess of liposomes

The concentration of viral protein was 10 μg/ml, which amounts to a lipid concentration of 2.8 μM. Liposome concentration was 25 μM lipid. The virus was labeled by the R18 probe as described under "Experimental Procedures." The calculations of % fusion activity were described in Ref. 6. Each point represents an average of 3 to 5 cases.

Liposome composition	Temperature	First round		Time of incubation at pH 5	Second round	
		I (%)	% fusion activity		*I* (%)	% fusion activity
	(°C)			*h*		
PC/PE	37	23.5 ± 4	26 ± 4	6	12.3 ± 2	14 ± 2
(2:1)	20	13.5 ± 1.5	15 ± 1.5	8	7.4 ± 1.5	8.5 ± 1.5
PC/PE/cholesterol (2:1:1)	37	24.7 ± 4	27 ± 4	8	15.2 ± 2	17 ± 2

TABLE II
Final intensity (I) levels and percents of virions fusing for influenza virus interacting with labeled liposomes

The concentration of viral protein was 20 μg/ml, which amounts to a lipid concentration of 5.6 μM. Liposome concentration was 5 μM lipid. The liposomes were labeled with NBD-PE and Rh-PE at a surface concentration of 0.6 mol% each. The calculations of % fusion activity were as described in Refs. 3 and 6. Each point represents an average of 3 to 5 cases. In second fusion rounds, the liposome concentration was the same, but the concentration of recovered virus was 28% (25–30%) of that in the first round. This accounts for the larger ratio between calculated % fusion activity and *I* (%) in the second fusion round.

Liposome composition	Temperature	First round		Time of incubation at pH 5	Second round	
		I (%)	% fusion activity		*I* (%)	% fusion activity
	(°C)					
PC/PE/PS/cholesterol (1:1:1:1)	37	20.3 ± 3	55 ± 5	15 min	8.8 ± 2	40 ± 10
	20	24.8 ± 3	65 ± 5	1 h	5.4 ± 3	24 ± 12
	4	24.2 ± 3	63 ± 5	7 h	5.8 ± 2.5	25 ± 10

by a phosphate assay. For the RET assay (24), LUVs were prepared with 0.6 mol% of both NBD-PE and Rh-PE incorporated in the lipid membrane.

Virus Labeling—Viral preparations were labeled with octadecylrhodamine B chloride as described previously (25, 26). The final self-quenching concentration of added probe corresponded to approximately 5 mol% of total viral lipid and that of ethanol was less than 1% (v/v). The mixture was incubated in the dark for 30 min at room temperature. R18-labeled virus was separated from noninserted fluorophore by chromatography on Sephadex G-75 (Pharmacia, Uppsala, Sweden) using 150 mM NaCl, 10 mM Hepes, pH 7.4, as elution buffer. The protein concentration of the labeled virus was determined by the Lowry assay.

Experiments with Liposomes—With the R18 assay fusion between labeled influenza virus particles (10 μg/ml viral protein) and unlabeled LUVs (25 μM) was monitored continuously by the relief of R18 fluorescence self-quenching (25, 26). Excitation was set at 560 nm and emission at 590 nm. With the RET assay, fusion between unlabeled influenza virus (20 μg/ml) and NBD/Rh-labeled (0.6 mol% each) lipid vesicles (5 μM) was followed by an increase of NBD fluorescence with 465 nm and 530 nm set in the excitation and emission monochromators, respectively (24).

The final incubation volume was always 1.5 ml in 135 mM NaCl, 5 mM sodium acetate, 5 mM Mes, 5 mM Hepes buffer. Following an initial 15-min incubation at pH 7.4, the virus-liposome suspension was acidified to pH 5.0, and fusion was monitored for the times and at the temperatures indicated below. The fluorescence scales were calibrated, such that the initial fluorescence of virus-liposome mixtures was set at 0% fluorescence. The value obtained by detergent lysis after each experiment was set at 100% fluorescence. With the R18 assay, Triton X-100, at a final concentration of 1% (v/v), was used. In the case of the RET assay, final fluorescence levels were obtained with $C_{12}E_8$ (1 mM).

All fluorescence measurements were performed in a Perkin-Elmer LS-50 luminescence spectrometer or in a SPEX Fluorolog spectrofluorimeter. The sample chamber was equipped with a magnetic stirring device, and the temperature was controlled with a thermostatted circulating water bath.

Separation of Unfused Virus Particles and Second Round Fusion Experiments—In "second round" fusion experiments, both assays were carried out as described above. However, following the appropriate times for fusion at different temperatures and at pH 5.0, the mixtures were then neutralized and overlaid on discontinuous sucrose density gradients. Gradients were then centrifuged at 250,000 × *g* in a Beckman ultracentrifuge to recover unfused virus particles from the gradient and thus separate them from LUVs and fusion products. Recovered virus particles were diluted in experimental buffer and their fusion activity toward LUVs of fresh batches was assayed using the same procedure (*i.e.* same liposome concentrations) as in the "first round," only in this case with a final addition of detergent. Control experiments showed that the use of virus concentrations below 10 μg/ml viral protein did not affect the final extent of fluorescence increases in the application of the R18 assay.

Experiments with Erythrocyte Ghosts—Human erythrocyte ghosts were prepared as described (27). Fusion activity of Sendai virus and influenza virus toward erythrocyte ghosts was monitored as a function of R18 dequenching, as in the case of the experiments using LUVs as target membranes. The experimental temperatures and respective times of incubation are mentioned under "Results." In all experiments, the concentration of viral protein in the first round was 2.5 μg/ml and that of erythrocyte ghost protein was 10 μg/ml. While Sendai virus fuses immediately at neutral pH, influenza virus was always allowed to bind to the target membranes for 15 min at pH 7.4, after which the medium was acidified to pH 5.

In first round experiments, an initial fluorescence reading (F_0) was taken before the onset of fusion. Following the incubation times required to obtain final extent levels of fluorescence, the mixture was neutralized (in the case of influenza virus) and ghosts were pelleted by centrifugation (12,000 × *g*, 0 °C), thus removing unbound virions. Fluorescence in the resuspended pellet (bound and/or fused virus particles) was measured before (F_t) and after the addition of detergent (F_∞). The percentage of fusion in this case only takes into account bound virions and was calculated according to the formula: % fusion = $100 \cdot (F_t - F_{02})/(F_\infty - F_{02})$ with $F_{02} = F_0 \cdot F_\infty / F_{\infty,0}$ where $F_{\infty,0}$ corresponds to the fluorescence intensity obtained when detergent was added directly to the initial virus preparation (with fluorescence F_0).

In second round experiments, an initial fluorescence reading (F_0) was also taken before the onset of fusion. Following final extent incubations, the mixture was neutralized (in the case of influenza virus), and ghosts were again pelleted by centrifugation. The pellet was then resuspended and layered on a sucrose density gradient, and unfused virus particles were recovered by ultracentrifugation as described for LUV experiments. These recovered virions were diluted in buffer, and their (quenched) fluorescence was measured (f_0). The fraction (β) of ghost protein added as a target membrane in this second round was calculated by the formula $\beta = f_0/F_0$ so as to maintain the same virus/ghost ratio in the second round. After mixing recovered virus with fresh ghosts, R18 dequenching was again monitored for the same time as in the first round (at neutral pH for Sendai virus; at low pH following a binding period of 15 min at pH 7.4 for influenza virus). When the incubation was concluded, ghosts were again pelleted by centrifugation and fluorescence in the resuspended pellet was measured before (f_t) and

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TABLE III

Final intensity (I) levels and percents of virions fusing for influenza virus labeled by R18 interacting with excess of liposomes: effect of the ganglioside G_{D1a}

In liposomes containing G_{D1a}, its content was 5 mol%. The molar ratio of PC to PE was 2:1. See Table I for details. The virus was from a different batch than in Table I. The first two and the last three experiments were on different days.

Liposome composition	Temperature	First round		Time of incubation at pH 5	Second round	
		I (%)	% fusion activity		*I* (%)	% fusion activity
	(°C)					
PC/PE	37	8.8 ± 2		15 min		
		34.6 ± 3	38.5 ± 3	6 h		
PC/PE/G_{D1a}	37	26.9 ± 4.5		15 min		
		54 ± 5	60 ± 5	6 h		
	—[a]	59.1 ± 2	65.7 ± 3	6 h		
	37	34.5 ± 4		15 min	26.2 ± 2	28.5 ± 2
		55 ± 4	61 ± 4	6 h	31.2 ± 2	34 ± 2
	20	50.4 ± 1	55 ± 1	1 h	22 ± 3	24 ± 3
		62 ± 1	69 ± 1	8 h	28.1 ± 3	31 ± 3
	—[a]	66 ± 3.5	73.1 ± 4	8 h		
	4	47.9 ± 1	53.5 ± 1	7 h		
		54.5 ± 2	60.5 ± 2	24 h		
(RET)	37[b]	24 ± 2	60.2 ± 5	6 h		
	20[b]	28.2 ± 2.8	70.8 ± 7	8 h		

[a,b] These cases employed another batch of virus for a comparison between the results with the R18 assay (a) and the RET assay (b). See Table II for details.

after (f_∞) the addition of detergent. The percentage of fusion in the second round was then calculated using the formulas mentioned above for first round experiments.

RESULTS

The first aim of the experiments was to test whether virions that do not fuse with the liposomes are capable of fusing with freshly added liposomes after their separation by prolonged centrifugation in sucrose gradients. The experiments also tested the effect of temperature on partial fusion activity of influenza virus. In order to avoid a possible criticism that exchange of the R18 probe can occur, we have employed two fusion assays. In the application of the R18 assay (25, 26), the labeled virus interacted with unlabeled liposomes in large excess. In this assay, probe dilution into liposomal membranes results in an increase in fluorescence intensity. Hence, if all the virions are capable of fusing, the increase in fluorescence intensity can approach 100% (6). In the application of the RET fusion assay (24) where N-NBD-PE and N-Rh-PE were incorporated in the bilayer of the liposomes, fusion of liposomes with the virus was monitored by an increase in the fluorescence intensity of NBD due to its dilution within the fusion products. According to the analysis (1), the extent of fluorescence intensity increase in the application of the RET assay is limited to 50% in the presence of a large excess of virions, whereas for a suspension consisting of equal numbers of fusion-active virions and liposomes, the extent of fluorescence increase is below 40%. Consequently, we employed the R18 fusion assay in fusion experiments with PC/PE (2:1) and PC/PE/cholesterol (2:1:1) liposomes (Table I), in which cases the percents of influenza virions capable of fusing could be estimated in the range of 20–40% according to the results of Ref. 4, whereas the RET assay was used in fusion studies with PC/PE/PS/cholesterol (1:1:1:1) where the percent of fusion-active virions was larger (Table II). We have also carried out a few experiments in which both assays were employed with the same liposome compositions (Table III). The tables also provide the estimated percents of virions capable of fusing in the first round. The calculations assumed that the binding of fusion-inactive virions was essentially irreversible (6).

Tables I and II show that at 37 °C influenza virus exhibits partial fusion activity toward the liposomes of the compositions used, the percents of virions capable of fusing varying from 26 to 55. In all three cases, the released virions were capable of fusing with freshly added liposomes. The fractions of virions capable of fusing in the second round varied from 14 to 40%. No further increase in fluorescence intensity at 37 °C was observed by prolonging significantly the incubation times beyond the indicated values. The explanation that is favored is that the virions that did not fuse in the first round happened to bind to the liposomes via sites on the viral membrane, in such a fashion that the virus could not fuse and that this binding was essentially irreversible.

A comparison of the results obtained by the application of both assays (Table III) illustrates that despite the significantly smaller extents of fluorescence increase with the RET assay, the estimated percents of virions capable of fusing in the first fusion round were similar, albeit somewhat larger with the R18 assay. This difference is indicative of a small degree of probe exchange with the R18 assay, but the trend is similar. It should be recalled that the lipid concentration of the liposomes was about 10-fold larger than that of the virions. The RET assay is less suitable for monitoring the percent of virus fusion in the second round due to a reduction in (unlabeled) virus concentrations. The calculated percents of second round fusion with the RET assay are probably underestimated.

Next we studied the effect of temperature on the percents of virions capable of fusing in the first and second rounds. To account for the slower kinetics of fusion at lower temperatures, we extended the incubation times at the lower temperatures. The fractions of virions which fused with PC/PE liposomes at lower temperatures were less than those at 37 °C, whereas for PC/PE/PS/cholesterol liposomes an apparent maximum was observed at 20 °C, but statistically it was not significant. In all cases, a fraction of the released virions fused in the second round.

It was of interest to examine whether partial fusion activity of influenza virus also occurs toward liposomes which include receptors for the virus, *e.g.* gangliosides (28). In Fig. 1, we illustrate the kinetics of fusion of influenza virus with PC/PE liposomes containing 5 mol% of the ganglioside G_{D1a}. At 37 °C, the initial rate of fusion is faster than at 20 °C, but after 2 min the extent of fusion at 20 °C exceeds that at 37 °C. This pattern is similar to earlier observations (20) and is explained by the fact that both fusion and inactivation rate constants increase with temperature (3, 13, 14). The final extents of fusion (Table III) demonstrate partial fusion activity of the virus toward

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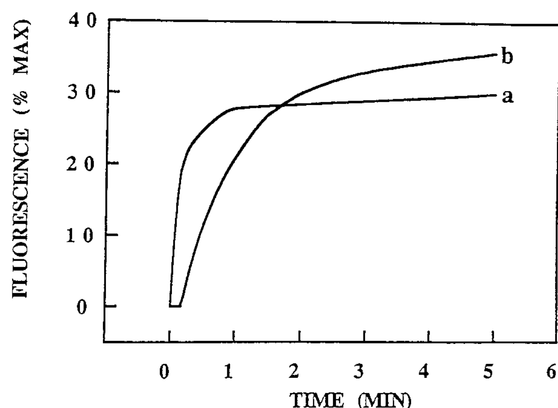

FIG. 1. **Fusion of R18-labeled influenza virus with ganglioside-containing liposomes.** R18-labeled influenza virus (10 μg/ml viral protein) was added to PC/PE (2:1) liposomes containing 5 mol% G_{D1a} (25 μM total lipid concentration) in a final volume of 1.5 ml. Following a 15-min incubation period at pH 7.4, the medium was acidified to pH 5.0 and fusion was monitored as a function of R18 fluorescence dequenching, as described under "Methods." Experiments were carried out both at 37 °C (*a*) and 20 °C (*b*).

PC/PE/G_{D1a} liposomes and the ability of the unfused virus to fuse when released after a long incubation time with the liposomes at pH 5.

The effect of temperature on the final extents of fusion of Sendai virus and influenza virus with erythrocyte ghosts is shown in Tables IV and V, respectively. In both cases, a decrease in temperature results in a significant decrease in final extents of fusion.

In both cases, the release of bound virus (after 20 h of incubation) resulted in its partial fusion activity toward erythrocyte ghosts, albeit reduced relative to the first round. It should be noted that the final extents of fusion in the second round (Tables IV and V) are underestimates, since the concentrations of particles were smaller, while keeping the ratios the same as in the first round. Control experiments (see Table V) demonstrate that lower particle concentrations result in smaller extents of fluorescence increase.

We performed another test for the hypothesis that a fraction of the virions bind in nonfusing conformations to the target membranes. In this test we used PC/PE/G_{D1a} liposomes. Following a 6-h incubation at pH 5 and 37 °C, where the extent of fluorescence reached equilibration, new R18-labeled virions were added to the medium (same amount as in the beginning of the experiment). We observed dequenching due to the freshly added virus (29.8% ± 5.8% after another 6 h). Addition of labeled virions to erythrocyte ghosts after 20 h also resulted in dequenching, 22% ± 3.8 with influenza virus and 17.7% ± 4.9 with Sendai virus. This result also rules out the possibility that the target membranes were inactivated by the incorporation of fusion-inhibiting molecules.

DISCUSSION

The phenomenon of partial fusion activity of viruses toward certain target membranes is distinct from the phenomenon of low pH inactivation. We have pointed out that Sendai virus (3, 5–7) and HIV-1 (8) exhibit at neutral pH partial fusion activity toward liposomes, but no inactivation has been observed. In the case of influenza virus, low pH inactivation did reduce the final extents of fusion with liposomes of a variety of compositions, but 100% of the inactivated virions fused with PS liposomes albeit at a very slow rate (4). It is remarkable that the virus released from the liposomes after long incubation at pH 5 could still fuse in the second round, but the fractions of virions that could fuse in the second round were 2/3 to 1/3 of those fusing in

TABLE IV
Final extents of fusion of Sendai virus with erythrocyte ghosts: effect of temperature on percent of virus fusion

Temperature	First round	Second round
37 °C	65.1 ± 3.9	31.1 ± 4.6
15 °C	42.8 ± 4.2	19.3 ± 6

TABLE V
Final extents of fusion of influenza virus with erythrocyte ghosts: effect of temperature on percent of virus fusion

Temperature	First round	Second round
37 °C	63.4 ± 3.6	17.1 ± 5
20 °C	36.6 ± 1	6.1 ± 3
15 °C	20.1 ± 2.5	ND[a]
37 °C (batch 2)	58.8 ± 4	
37 °C (batch 2) (2-fold dilution of virions and cells)	39.2 ± 3.2	

[a] ND, not determined.

the first round. In the case of Sendai virus (3), the corresponding reduction was 5/6 to 2/3. Thus, most of the reduction in the percent of fusion-active influenza virions is due to low pH inactivation, and part might be due to the loss of activity resulting during the sequence of binding and release that might have impaired the structure and function of viral glycoproteins.

In experiments with erythrocyte ghosts, the fractions of Sendai and influenza virions that could fuse in the second round were about 1/2 and 1/6 to 1/4, respectively. Again, the reduced fraction in the case of influenza virus might be largely due to low pH inactivation and partly due to dilution of particle concentrations in the second round.

Our experiments eliminate the possible explanation for partial fusion activity that the virus preparation is heterogeneous, in the sense that a certain fraction of the virions cannot induce membrane destabilization of membranes of certain compositions, although binding can occur. The experiments demonstrate that the virions that did not fuse in the first round are not necessarily incapable of fusing, since upon their release a certain fraction can fuse. Thus we tend to accept the proposal that partial fusion activity is a manifestation of heterogeneity at the viral surface. It has been formulated (3) that if virus binding to a liposome does not occur at an "active" (viral) site, fusion will not occur. If this binding is essentially irreversible under the given conditions, as was indeed found for Sendai virus (6), then all of the virions may be bound in the presence of large excess of liposomes (Tables I and III), but a certain fraction will remain unfused. If such an attached virus is released from the liposome, it has the same chance as any other virus in the population to fuse with liposomes by forming an attachment via an active site.

The results of fusion with PC/PE liposomes without gangliosides demonstrate a reduction in the final extents at lower temperatures than 37 °C and, consequently, a reduction in the percent of fusing virions. This result is particularly interesting in view of the fact that the rate of low pH inactivation is dramatically reduced upon lowering the temperature (3, 13, 14, 20). Truly, a situation can arise where the enhanced rate of low pH inactivation at higher temperatures can result in a reduction in the final extents of fusion. Such differences can also reflect differences in viral strains. We propose two explanations which are not mutually exclusive. The enhanced percentage of virions that can fuse with PC/PE liposomes and erythrocyte ghosts at 37 °C than at 20 °C or 4 °C, might be due to the larger tendency of bound colloidal particles to dissociate upon elevation of the temperature (29). Thus, the dissociated virions have a second chance to bind via sites which promote fusion. It was

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found (30) that the final extents of fusion of Sendai virus with didodecyl phosphate vesicles increase with temperature. The detailed analysis of fusion kinetics indicated indeed a significant increase in the rate constant of dissociation with temperature. In addition, at higher temperatures the viral glycoproteins might arrange themselves in a configuration which is more optimal for fusion.

The concept that site heterogeneity in the viral surface may manifest itself in partial fusion activity may be generalized in the sense that viral glycoproteins or peptides may form several intermediate states in the target membrane (31, 32). Again, if certain intermediate states which do not lead to completion of the fusion process are formed irreversibly, then a fraction of the virions would remain unfused. Evidence for essentially irreversible association of peptides in the target membrane has recently been presented in the case of HIV_{arg} (33).

We have pointed out that in many cases influenza and Sendai virions have partial fusion activity toward liposomes but not cells. It is possible that, when the virus binds to the cellular surface via an inactive site on the virus, or alternatively an inactive intermediate state is created in the vicinity of the attachment site, there is geometrically a chance of forming a contact with the cellular surface via another active site.

In the experiments conducted in this study, the mechanism of partial fusion activity of these viruses toward erythrocyte ghosts is not necessarily the same as in the case of liposomes, since the percent of fusing virions would be increased by increasing ghost concentrations, whereas with the liposomes (Tables I and III) any further increase in liposome concentrations would increase the final extents of fusion only slightly. Hence, the major effect could be due to saturation of the ghosts' membranes with respect to viral glycoproteins (3). According to this reasoning, the dramatic decrease in the final extents of fusion at lower temperatures might be the effect of temperature on the capacity of the ghosts' membranes to incorporate the viral glycoproteins. However, there are cases of partial fusion activity of Sendai virus and influenza virus toward erythrocyte ghosts in large excess (7, 15) and, more dramatically, a very small fraction of bound HIV-1 particles fuse with erythrocyte ghosts (8) or $CD4^+$ target cells (16). Our results demonstrate that influenza or Sendai virions released from erythrocyte ghosts after 20 h of incubation can still fuse with the target cells under the same conditions, similarly to the liposome experiments. This result might imply that unfused virions bound to a cellular membrane may have a certain probability of fusion with similar target membranes following their release.

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