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Kinetic modeling of Sendai virus fusion with PC-12 cells

Effect of pH and temperature on fusion and viral inactivation

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We have studied the fusion activity of Sendai virus, a lipid-enveloped paramyxovirus, towards a line of adherent cells designated PC-12. Fusion was monitored by the dequenching of octadecylrhodamine, a fluorescent non-exchangeable probe. The results were analysed with a mass action kinetic model which could explain and predict the kinetics of virus – cell fusion. When the temperature was lowered from 37°C to 25°C, a sharp inhibition of the fusion process was observed, probably reflecting a constraint in the movement of viral glycoproteins at low temperatures. The rate constants of adhesion and fusion were reduced 3.5-fold and 7-fold, respectively, as the temperature was lowered from 37°C to 25°C. The fusion process seemed essentially pH-independent, unlike the case of liposomes and erythrocyte ghosts. Preincubation of the virus in the absence of target cell membranes at neutral and alkaline pH (37°C, 30 min) did not affect the fusion process. However, a similar preincubation of the virus at pH = 5.0 resulted in marked, though slow, inhibition in fusion with the fusion rate constant being reduced 8-fold. Viral preincubation for 5 min in the same acidic conditions yielded a mild inhibition of fusogenic activity, while preincubation in the cold (4°C, 30 min) did not alter viral fusion activity. These acid-induced inhibitory effects could not be fully reversed by further viral preincubation at pH = 7.4 (37°C, 30 min). Changes in internal pH as well as endocytic activity of PC-12 cells had small effect on the fusion process, thus indicating that Sendai virus fuses primarily with the plasma membranes.

Membrane fusion is a key event spanning a wide variety of biological processes. Although the molecular mechanisms involved remain largely obscure, the introduction of simple model systems has cast considerable light on some of the specific fusion requirements. In this regard lipid-enveloped viruses have proven particularly effective tools. These viruses infect cells following a fusion event, usually mediated by only a few viral membrane proteins. The study of virus fusion with cellular membranes is of biological relevance, yet it is simpler than intracellular membrane fusion (for reviews see [1 – 3]).

Sendai virus, a paramyxovirus, is thought to fuse with a target cell plasma membrane at neutral pH by the combined action of two viral envelope glycoproteins, one involved in virus – cell binding and one specific for fusion [4]. The protein designated HN exhibits hemagglutinin and sialidase activity and mediates the initial virus – target membrane binding. However, there is an indication that, at least in the case of certain liposomes, the fusion process itself involves penetration of the target membrane bilayer by the fusion protein (F) [5].

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Abbreviations. R18, octadecylrhodamine B chloride; $C_{12}E_8$, octaethyleneglycol dodecyl ether.

Extensive studies on the fusion activity of Sendai virus towards liposomes [6 – 8] and erythrocyte ghosts [9 – 11] have been published. Some work has also focussed on the infection of living cells [2, 12].

In the work presented here we report fusion of Sendai virus with cultured cells. A cell line established from rat adrenal pheochromocytoma, and designated PC-12, was used for this purpose [13, 14]. We studied the fusion of Sendai virus with these adherent cells in suspension by employing a fluorescence assay that can monitor fusion continuously. A mathematical analysis based on a mass action kinetic model was used to determine the adhesion and fusion rate constants. We have applied the model to characterize the effect of temperature and low pH preincubation of the virus, or inactivation, on its fusion with the cells. Other issues addressed have been the pH dependence and the contribution of endocytosis to the overall fusion events in the interaction of Sendai virus with PC-12 cells.

EXPERIMENTAL PROCEDURES

Virus

Sendai virus (hemagglutinating virus of Japan), Z strain, was obtained from Dr. Dick Hoekstra (University of Groningen). The virus was grown for 72 h in the alantoic cavity of

10-day-old embryonated eggs, purified by differential centrifugation as described elsewhere [15] and stored at −70°C in phosphate-buffered saline.

Cells

PC-12 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown in RPMI 1640 medium containing 25 mM Hepes, supplemented with 10% foetal calf serum and 5% heat-inactivated horse serum. The cells were grown in T-75 flasks up to a cell density of $1-1.5\times10^6$/ml under a 5% CO_2 atmosphere in a Ferma Scientific incubator.

The cells were harvested by centrifugation at 180 *g* for 8 min at room temperature, washed twice in phenol red-free RPMI 1640 supplemented with 25 mM Hepes and resuspended in a saline buffer containing 130 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 15 mM Hepes pH 7.4.

The cells, which form clusters, were dispersed by several passages through a 22-gauge syringe and then counted in a hemocytometer. Cell viability was determined by trypan blue exclusion and was routinely above 90%. This viability remained constant throughout the experiments. The cells were then transferred to fluorometer quartz cuvettes in the desired final density. Cell−cell aggregation was avoided by continuous stirring.

Virus labeling

The virus was labeled with octadecylrhodamine B chloride (R18, Molecular Probes Inc., Eugene, OR) as described by Hoekstra et al. [9]. A 10-μl aliquot of a 4 μmol/ml ethanolic fluorophore solution was injected under vortex mixing into a viral suspension containing 2 mg viral protein/ml. The final concentration of added probe corresponds to approximately 5 mol % of total viral lipid and that of ethanol was 1% (by vol.). The mixture was incubated in the dark for 0.5−1 h at room temperature. R18-labeled virus was separated from noninserted fluorophore by chromatography on Sephadex G-75 (Pharmacia, Uppsala, Sweden) using 10 mM Tes, 150 mM NaCl, pH 7.4, as elution buffer. The protein concentration of the labeled virus was determined by the Lowry assay.

Fusion of R18-labeled Sendai virus with PC-12 cells

Fusion, monitored continuously with the fluorescence assay as described elsewhere [9, 16], was initiated by rapid injection of R18-labeled virus into a cuvette containing the cell suspension. The final incubation volume was always 2 ml. The fluorescence scale was calibrated such that the initial fluorescence of R18-labeled virus and cell suspension was set at 0% fluorescence. The value obtained by lysing the virus and cellular membranes after each experiment with octaethyleneglycol dodecyl ether ($C_{12}E_8$; Calbiochem, San Diego, CA) at the final concentration of 3.15 mM was set at 100% fluorescence.

Fluorescence measurements were performed in a Perkin-Elmer LS-5B luminescence spectrometer with excitation at 560 nm and emission at 590 nm, using 5-nm and 10-nm slits, respectively, in the excitation and emission monochromators. The sample chamber was equipped with a magnetic stirring device and the temperature was controlled with a thermostatted circulating water bath. Unless indicated otherwise, the experiments were carried out at 37°C, pH 7.4.

Binding and cell association

Fluorescently labeled Sendai virus was incubated with PC-12 cells in a final volume of 2 ml saline buffer (see above) with continuous stirring. The incubation time was 15 min. Binding was carried out at 4°C and cell association was determined at 37°C. Mixtures were then transferred to polypropylene tubes and centrifuged either at 4°C (binding) or room temperature (cell association) for 8 min at 180 *g*. Fluorescence was measured in the pellet and supernatant after the addition of $C_{12}E_8$ (3.15 mM) to determine the fraction of bound, or cell-associated virus, and free virus, respectively.

Enzymatic treatment

Trypsin treatment was performed by incubating 2 μg viral protein with 14 μg trypsin in a final volume of 114 μl for 45 min at 37°C. The reaction was terminated by adding a twofold excess of trypsin inhibitor. For proteinase K treatment, 2 μg of viral protein was incubated for 30 min at 37°C at a final enzyme concentration of 0.05 mg/ml. Following this incubation, the virus was added to the fluorometer cuvette containing the cells at 37°C. In the fusion experiments the proteinase K concentration was reduced 20-fold.

Other procedures

PC-12 cells (3.5×10^6 cells) were incubated with 0.1% (mass/vol.) sodium azide for 25 min at 37°C (in a total volume of 1.9 ml) with continuous stirring. This procedure has been described to reduce cell endocytic activity [17]. Incubation of PC-12 cells (3.5×10^6 cells) with either 20 mM NH_4Cl or with 9 μM monensin (15 min, 37°C, total volume 1.9 ml and continuous stirring) was carried out to increase the pH in intracellular acidic compartments [18, 19]. Following the incubations, labeled Sendai virus (2 μg viral protein) was added to the cells and fusion was monitored in each case as described above.

PC-12 cells were sized electronically using a Coulter Counter model ZM and a Channelyzer 256. The median cell diameter averaged 14 μm.

Analysis of data

The analysis was essentially as described in Nir et al. [11], with certain modifications as described below. We have employed three parameters: C ($M^{-1}\cdot s^{-1}$), the rate constant of viral adhesion to the cells; $f(s^{-1})$, the rate constant of the actual fusion of an adhered virus particle; $D(s^{-1})$, the dissociation rate constant.

The analysis of fusion kinetics and fluorescence intensity increase requires a knowledge of another parameter; N_f, the number of virus particles that can fuse with a single cell; this can be deduced from the final extents of fluorescence increase. The experiments were designed to avoid an excessive ratio of virus particles/cell. We employed 0.5−5 μg viral protein/ml, which amounts to 0.74−7.4 pM particles [11], and used 2×10^6 or 3.5×10^6 cells/2 ml. Thus the number of virions/cell was between 250−4400. The analysis of kinetics was limited to cases in which less than 500 virions have fused per cell. In such cases the calculations were relatively insensitive to 50% variation in the value of N_f, which was about 1500.

The outcome of the measurements of the viral fraction associated with the cells [10] provided another test for the model calculations and enabled to reduce the uncertainty in

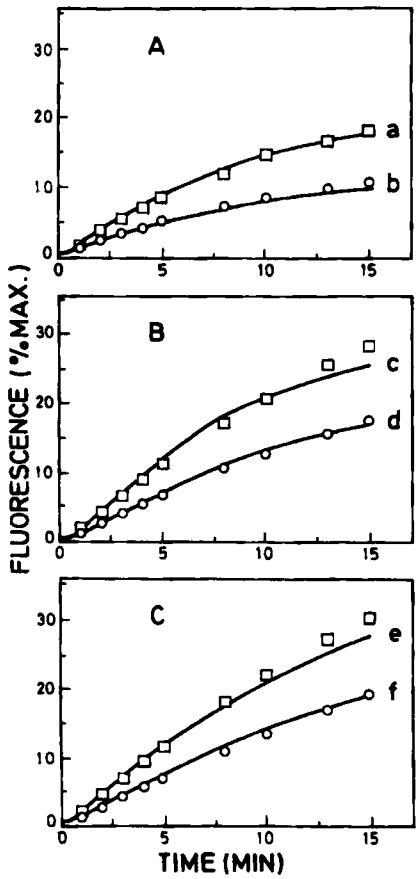

Fig. 1. Fusion of Sendai virus with PC-12 cells: effect of virus and cell concentrations. R18-labeled Sendai virus was added to PC-12 cells and fusion was monitored at pH 7.4 and 37°C as a function of R18 fluorescence dequenching. (A) 5 μg viral protein/ml; (B) 1 μg viral protein/ml; (C) 0.5 μg viral protein/ml. Curves (a, c and e) 3.5×10^6 cells/2 ml; (b, d and f) 2×10^6 cells/2 ml. Also shown are the values calculated for both cell concentrations in each case using the mass action kinetic model. (□) 3.5×10^6 cells; (○) 2×10^6 cells.

the values of the parameters C, f, D [11, 20, 21]. The procedure of prebinding the virus to the cells in the cold was not employed because of a delay in fusion activity of the virus upon dilution of the suspension into warm buffer [21], and in order to avoid massive aggregation of cells. The current program includes an extension that enables to consider explicitly viral inactivation [22]. In most cases, a significant cut in computation times could be achieved by employing the expressions in Bentz et al. [20]. For very small values of $f < (D \approx 0.005\ s^{-1})$ those approximate expressions could not be employed. Ultimately all final values were generated by numerical solutions [11].

RESULTS

Fusion of virus with target cell membranes

We have used the R18 fluorescence dequenching assay to monitor fusion events between lipid-enveloped Sendai virus particles and PC-12 cells. Fusion was quantified by continuous fluorimetric measurements. Fig. 1 illustrates the kinetics of fluorescence intensity increase for two cell concentrations and three viral concentrations. The extent of fusion, expressed as percentage of maximum probe dequenching, increased with cell concentration.

The model calculations [11] yielded good simulations for curve (a) in Fig. 1 and predictions for the other curves; the fusion parameters (adhesion, fusion and detachment rate constants) are presented in Table 1 (batch 1). The calculations also account for the amount of virus which is associated with the cells. It may be noted that 5 μg viral protein/ml amounts to 7.4 pM [8] whereas 3.5×10^6 cells/2 ml corresponds to 2.5 fM. Thus, 10% fusion in this case corresponds, on average, to 300 virions fused/cell. The calculations which yield the distribution of adhesion-fusion products indicate that the distribution is rather narrowly centered around the average.

It can be noted that higher virus concentrations yield lower extents of fusion (Fig. 1). This fact can be explained by the presence of a limited number of fusion sites on the cell surface, which becomes saturated with an increase in the number of virus particles, i.e. at higher concentrations the percentage of virus involved in actual fusion becomes lower.

The system was further characterized using long incubation times (5 h) of the virus with the cells. The results of fluorescence increase (with 3.5×10^6 PC-12 cells/2 ml and 1 μg viral protein/ml) indicate that most of the virions are capable of fusing with the cells at 37°C and neutral pH. This can be deduced by the final level attained, which was 77%. The results with 2×10^6 cells/2 ml and 5 μg viral protein/ml yield a certain lower bound on the number of virions that can fuse with a single cell. The viral concentration in this case was 7.4 pM (see above), whereas the concentration of cells was 1.7 fM; the number of virions/cell is 4350 of which about 1500 have fused after 5 h. This value is obtained if we ignore any fluorescence intensity increase due to probe exchange rather than fusion (see below). On the other hand, if viral fusion does not result in complete dequenching the above estimate can be increased. It was shown [10, 11, 21] that about 100–200 virions can fuse/single erythrocyte ghost, whereas about 1500 virus particles can adhere to it in the cold [10]. Our results do not suggest such a big ratio between binding and fusion capacity. The ratio of 4350:1500 (binding) and 1500:100 (fusion) may reflect, in part, the ratio between surface areas of a PC-12 cell and of an erythrocyte ghost.

Temperature effect

Sendai virus fusion with PC-12 cells was dramatically reduced when the temperature was lowered from 37°C to 25°C (Fig. 2). A slower initial fusion rate, as well as lower fusion extents, were observed at 25°C. This fact is possibly due to a lower mobility of viral glycoproteins [21] and could be correlated with a decrease in the adhesion and fusion rate constants calculated for this temperature (Table 1, batch 2).

pH effect on fusion rates and inactivation

Adding virus to cells at different extracellular pH values did not produce significant differences. Although a slight optimal peak was found at neutral pH, the process seems largely pH-independent (results not shown).

However, a preincubation of the virus at pH = 5.0 (37°C, 30 min) in the absence of target cell membranes resulted in a large extent of fusion inhibition (Fig. 3). This inhibition amounts to about twofold and eightfold reduction in the rate constants of adhesion and fusion, respectively (see Table 1, batch 3, 30 min). Under the same conditions, viral preincubation for 5 min yielded only a mild inhibition of the fusion process (Fig. 3; Table 1, batch 3, 5 min), while preincubation for 1 min did not alter fusion activity (not shown). These acid-

Table 1. Rate constants describing the kinetics of Sendai virus fusion with PC-12 cells. Fusion was monitored by R18 dequenching as described in the Experimental Procedures. In all cases 1 μg/ml of viral protein was incubated with 3.5×10^6 PC-12 cells. For batch 1, fusion was monitored at pH = 7.4 and 37 °C. For batch 2, the pH was 7.4 and the temperature was either 37 °C or 25 °C as shown below. For batch 3, the experiments were carried out at pH = 7.4 and 37 °C, but the virus was first incubated at pH = 5.0 in the absence of cells for 5 min and 30 min (see below). Controls for batch 3 yielded the same rate constants as for batch 1. Analysis of data was carried out as described in Experimental Procedures. The estimated uncertainties for f, C and D were 30%, 20% and 50%, respectively. See also Hockstra et al. [21] for statistical tests. Each batch refers to a different passage of cells, all belonging to the same source.

Batch	Conditions			Adhesion rate constant, C	Fusion rate constant, f	Detachment rate constant, D
	pH	temp.	preincubation time			
		°C	min	$M^{-1} \cdot s^{-1}$	s^{-1}	
1.	7.4	37	–	1.8×10^{11}	0.03	0.005
2.	7.4	37	–	1.4×10^{11}	0.03	0.005
	7.4	25	–	4.0×10^{10}	0.0045	0.001
3.	5.0/7.4	37	5	1.25×10^{11}	0.01	0.005
	5.0/7.4	37	30	8.0×10^{10}	0.004	0.005

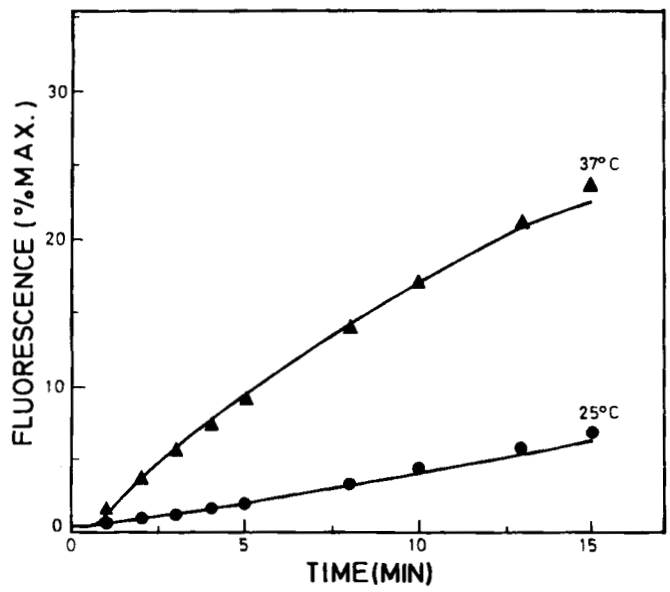

Fig. 2. Effect of temperature on the fusion of Sendai virus with PC-12 cells. R18-labeled Sendai virus (1 μg viral protein/ml) was added to 3.5×10^6 PC-12 cells/2 ml and fusion registered continuously at the indicated temperature values and pH 7.4. Calculated values using the mass-action kinetic model are also depicted for both 25 °C (●) and 37 °C (▲).

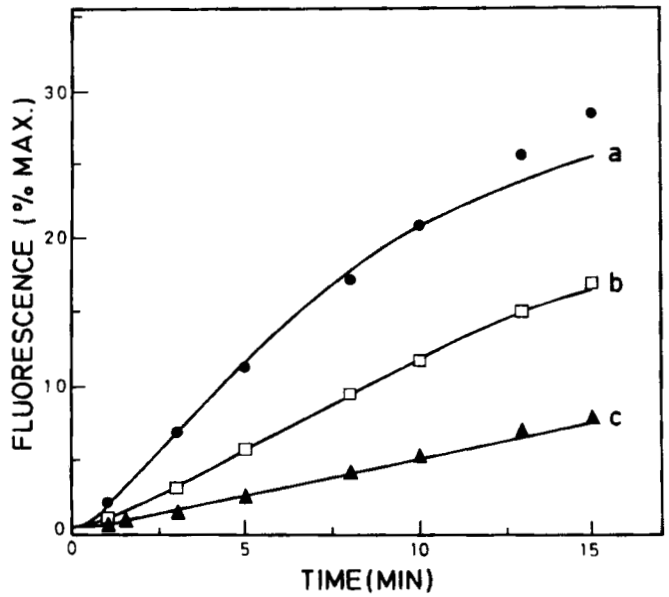

Fig. 3. Effect of preincubation of Sendai virus at low pH on its fusion with PC-12 cells. R18-labeled Sendai virus (2 μg viral protein) was preincubated at pH = 5.0 and 37 °C in the absence of target membranes and added to 3.5×10^6 PC-12 cells in a final volume of 2 ml at pH = 7.4 and 37 °C. Curve (a), control (no preincubation); (b) 5-min preincubation; (c) 30-min preincubation. Calculated values using the mass-action kinetic model are also depicted for curves a (●), b (□) and c (▲).

induced inhibitory effects were not due to viral aggregation as determined by light scattering (not shown) and could not be fully reversed following further viral preincubation at pH = 7.4 (37 °C, 30 min).

On the other hand, when preincubation took place in the cold (4 °C, 30 min, pH = 5.0) no alteration in the viral fusion activity was evident. It is also interesting to note that viral binding to the cells (4 °C, 15 min) was unaffected by acid pretreatment (not shown).

Preincubation of Sendai virus at pH 9–10 and 37 °C produced only a slight inhibitory effect (2–3% of maximal probe dequenching after 15 min), while the same procedure carried out at neutral pH (corresponding to viral pre-heating) had not effect whatsoever (not shown).

Possible viral fusion with endocytotic acidic compartments was assessed by preincubation of the cells with monensin and ammonium chloride (agents known to increase the pH in intracellular compartments [18, 19]) and azide (known to reduce endocytosis [17]). These treatments did not affect virus–cell fusion (not shown). Therefore, our results indicate that Sendai virions interact mainly with the plasma membranes of PC-12 cells at neutral pH.

Fusion versus exchange

Some experiments were designed to show whether fusion-independent probe exchange could occur between the viral

Table 2. Effect of inhibitors and excess unlabeled virus on the fluorescence increase and cell association of R18-labeled Sendai virus incubated with PC-12 cells. R18-labeled Sendai virus (2 μg protein) was pretreated with proteinase K or trypsin (as described under Experimental Procedures) or added simultaneously with unlabeled virus (100 μg protein) to 3.5×10^6 PC-12 cells (final volume 2 ml) and fusion (fluorescence increase) was monitored as described (pH = 7.4, 37°C). In each case the percentage of cell association was calulated by measuring the fluorescence in the supernatant (nonbound virions) and in the pellet (cells and bound virions) after addition of detergent as described; all results are presented for $t = 15$ min.

Conditions	Flurorescence increase (cf. maxm.)	Inhibition	Cell association (cf. maxm)	Inhibition
	%			
Control	22.8	–	39.9	–
Proteinase K	0.0	100.0	28.8	27.8
Trypsin	13.8	39.5	27.3	31.6
Excess (50-fold) of unlabeled virus	10.0	56.1	30.6	23.3

envelope and the target cell plasma membrane, when both membranes are in close contact. The purpose of these experiments was therefore to lower fluorescence increase due to viral fusion, i.e. R18 dequenching, while maintaining a reasonable percentage of cell-associated fluorescence (i.e. viral binding). Both limited proteolytic cleavage of viral particles (in an attempt to cleave preferentially the F protein), as well as competition between labeled virions and an excess of unlabeled virions (reducing the possibility of labeled virions attaching to a fusion site in the cell membrane) were tried.

The results obtained (Table 2) show that fluorescence increase due to R18 dequenching (fusion) could be sharply reduced (or abolished) while maintaining significant levels of cell-associated fluorescence (binding). Thus probe exchange between non-fusing bound virions and target cells does not contribute much to probe dilution.

DISCUSSION

The fusion of Sendai virus with PC-12 cells is mostly limited to its fusion with plasma membranes since inhibition of endocytosis did not affect the fluorescence intensity increase during an hour of incubation of the virus with the cells. It may be added that acidification of the endosomes plays a minor role on the fusion of Sendai virus, since fusion of the virus is almost independent of pH, although it is slightly favored at neutral pH.

The results in Figs 1 and 2 illustrate that the mass action kinetic model can simulate and predict the kinetics of Sendai virus fusion with PC-12 cells. A comparison of curves a with b, c with d, and e with f in Fig. 1 illustrates that an approximate twofold increase in the number of cells results in a significant increase in the overall rate of fusion of the virus with the cells. A comparison between the curves in Fig. 1 A and 1 C illustrates the effect of a tenfold variation in virus concentration. According to the model, the initial increase in fluorescence intensity or fusion is independent of the concentration of the virus. This result is indeed observed. At later times, the curves in Fig. 1 A show a significantly smaller extent of fluorescence increase than those in Fig. 1 B and C, due to the occupation of adhesion and fusion sites on the plasma membrane of the cells. We note that the numbers of virions/cell in curves a, c and e are 2500, 500 and 250, respectively. In both Figs 1 and 2 the calculated values overestimate the experimental values at later times, e.g. 15 min. We interpret this result to be due to cell–cell aggregation that results in a reduction in the cellular surface area available for the virus. We note that this effect is more extensive in suspensions with larger numbers of cells. Indeed, we observed that PC-12 cells, which are slightly adherent (they grow in clusters), tend to self-aggregate, but we have not quantitated this cell aggregation. This phenomenon has been extensively studied with other cells [22–24].

A time lag of the order of several seconds to about a minute has been frequently observed in virus-cell fusion experiments (see Fig. 1). Our detailed analysis reveals that at 37°C the time lag is only apparent and is fully explained in the framework of the model as due to the time required for adhesion followed by actual fusion. In the hypothetical case that all of the virus is prebound to the cells and fusion starts at time $t = 0$, the fraction of fused virus would be initially given by ft [11], in which f is the fusion rate constant. Thus, by taking $f = 0.03\ s^{-1}$, the time required for observing 2% fluorescence increase would be about 0.5 s. In the other extreme case in which fusion is assumed to occur instantaneously following adhesion, the initial fraction of fused virus would be CGt [11], in which C is the adhesion rate constant and G is the molar concentration of cells. In this case using $C = 1.8 \times 10^{11}\ M^{-1} \cdot s^{-1}$ and $G = 3$ fM gives that the time required for 2% fusion would be 40 s. In the general case, the delay is due to both adhesion and fusion. The apparently longer delay seen in Fig. 2 at 25°C is mostly due to a significant reduction in the fusion rate constant. No apparent delay has been previously seen in the fusion of Sendai virus with liposomes [6–8, 25, 26]. However, a genuine time lag was noted in the case of Sendai virus preincubated with erythrocyte ghosts in the cold [21] and was explained as due to constraints on the mobility of viral glycoproteins at low temperatures.

The results of the analysis enable us to compare the fusion characteristics of Sendai virus with PC-12 cells with those of its fusion with other target membranes, such as erythrocyte ghosts and liposomes. The fusion rate constant obtained here is within the range of values previously obtained for Sendai virus fusion with erythrocyte ghosts [11, 21], which is somewhat below the values found for fusion with liposomes [8], but the liposomes employed in that study, cardiolipin [1,3-bis(phosphatidyl)-*sn*-glycerol], cardiolipin/phosphatidylcholine and phosphatidylserine, were more fusogenic than those composed of phosphatidylcholine/phosphatidylethanolamine and gangliosides [7].

We have pointed out that all virus particles demonstrated fusion activity towards PC-12 cells, similarly to their fusion with erythrocyte ghosts. The number of virus particles that can fuse with a single PC-12 cell is about 1500, whereas about 100–200 virus particles fuse with an erythrocyte ghost [10, 11]. If we consider that the radii of erythrocyte ghosts and PC-12 cells are 3.5 μm and 7 μm, respectively, then it follows that the number of virions that can fuse per PC-12 cell is about twice the value corresponding to the ratio of areas. This comparison implies that the plasma membrane of a PC-12 cell bears similarity to that of an erythrocte ghost with respect to its fusion characteristics towards Sendai virus at neutral pH. There is a difference, however, in terms of pH dependence. The rate of fusion of Sendai virus with erythrocyte ghosts is optimal at neutral pH [2, 9], whereas in the case of PC-12 cells

the pH dependence is diffuse. We note that the fusion activity of Sendai virus towards acidic liposomes was significantly enhanced upon lowering the pH [6–8].

The adhesion rate constants of Sendai virus with PC-12 cells and erythrocyte ghosts are $(1-2)\times 10^{11}$ and $(1-4)\times 10^{9}$ $M^{-1}\cdot s^{-1}$, respectively. According to Berg and von Hippel [27], C values increase approximately linearly with the ratio between the radii of particles. However, the above ratio between C values is beyond the corresponding ratio of radii. Consequently, it can be stated that the plasma membrane of a PC-12 cell enables Sendai virus particles a significantly faster adhesion relative to that of an erythrocyte ghost. The values we found for the parameter C are close to the upper values in diffusion-controlled processes [28].

It has been reported [6, 9, 12] that Sendai virus looses part of its fusion activity towards cells or liposomes, following an incubation of the virus alone at pH lower than 5–6. Here this effect is quantified for the first time for the case of Sendai virus. In the case of influenza virus fusing with liposomes [29, 30], where binding of liposomes to the virus was essentially unaffected, the analysis considered just the variation of the parameter f. Here we found about eightfold reduction in f, and twofold reduction in C (see Table 1, batch 3) following 30 min of incubation of the virus alone at pH 5. An extensive treatment of the equations employed in the analysis of viral inactivation has been presented by Nir et al. [22, 29]. For brevity, we consider here a decay of the fusion rate constant during incubation of the virus at low pH according to:

$$f(t) = f(0)\exp(-gt), \qquad (1)$$

in which g is a decay rate constant. Thus substituting t = 1800 s in Eqn (1) yields $g = 0.001\ s^{-1}$. This means that incubation of the virus at pH 5 (37°C) for 1 min and 5 min should yield, respectively, 6% and 26% reduction in f. Consequently we can anticipate no inactivation following 1 min of viral incubation at pH 5, whereas a small degree of inactivation is expected after 5 min. This prediction was indeed observed (Fig. 3). The decay rate constant found for Sendai virus is more than tenfold smaller than that found for influenza virus [22, 29, 30]. Similarly to the studies on influenza virus [31, 32] we have not seen loss of fusion activity of Sendai virus following 30 min of incubation of the virus at pH 5 in the cold. Hence, it could be stated, as in the case of influenza virus [31, 33], that clustering of viral glycoproteins within the viral envelope is responsible for the low-pH inactivation. This clustering is inhibited in the cold. However, the analysis and the observations cannot rule out other possible mechanisms of low-pH inactivation of the virus, such as certain conformational changes or spatial rearrangements of the viral glycoproteins, which are also inhibited in the cold. The fusion activity of Sendai virus towards PC-12 cells following its preincubation at low temperature in an acidic medium was different from what has been described for erythrocyte ghosts [9]. In the latter case, low-pH viral inactivation was shown to be temperature-independent. On the other hand, viral preincubation at alkaline pH has also been described either to reduce [9] or to enhance Sendai virus activity [12]. These observations point to a probable influence of the target membrane on the viral behavior and on the characteristics of the fusion process.

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