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Fusion of Sendai virus with human HL-60 and CEM cells: different kinetics of fusion for two isolates

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The kinetics of fusion of Sendai virus (Z strain) with the human promyelocytic leukemia cell line HL-60, and the human T lymphocytic leukemia cell line CEM was investigated. Fusion was monitored by fluorescence dequenching of octadecylrhodamine (R-18) incorporated in the viral membrane. For one virus isolate (Z/G), the overall rate of fusion (at 37 °C) increased as the pH was lowered, reaching a maximum at about pH 5, the lowest pH tested. For another isolate (Z/SF) the rate and extent of fusion were lower at pH 5 than at neutral pH. Lowering the pH from neutral to 5 after several minutes of incubation of either isolate with HL-60 cells resulted in an enhanced rate of fluorescence dequenching. Nevertheless, experiments utilizing NH_4Cl indicated that fusion of the virus with cells was not enhanced by the mildly acidic pH of the endosome lumen. Analysis of the kinetics of fusion by means of a mass action model resulted in good simulation and predictions for the time-course of fusion. For the isolate which showed maximal fusogenic activity at pH 5, the rate constant of fusion (approx. 0.1 s^{-1}) at neutral pH was in the range found previously for virus-liposome fusion, whereas the rate constant of adhesion was close to the upper limit for diffusion-controlled processes ($1.4 \cdot 10^{10}$ M^{-1} s^{-1}). However, for the other isolate (Z/SF) the rate constant of fusion at neutral pH was very small (< 0.01 s^{-1}), whereas the rate constant of adhesion was larger ($\geq 2 \cdot 10^{10}$ M^{-1} s^{-1}). Lowering the temperature decreased the fusion rate. Experiments involving competition with excess unlabeled virions indicated that not all binding sites for Sendai virus on HL-60 cells are fusion sites. The virus fusion activity towards HL-60 cells at neutral pH was not altered significantly by pre-incubation of the virus at pH 5 or 9, in contrast to earlier observations with liposomes and erythrocyte ghosts, or results based on erythrocyte hemolysis or cell-cell fusion.

Introduction

Sendai virus (hemagglutinating virus of Japan) belongs to the paramyxovirus family, and is believed to infect its host cells by fusion of its envelope with the cellular plasma membrane [1–4]. The viral envelope contains the F (fusion) and HN (hemagglutinin/neuraminidase) proteins, both of which are thought to be involved in the overall fusion process, consisting of the steps of adsorption to the cell membrane, destabilization of the target and viral membranes and merging of the two membranes. Reconstitution of these proteins in liposomes has revealed that optimal fusion with the plasma membrane is obtained at a ratio of 2:1 F:HN [5]. Okada [2] has suggested that an optimum conformation of the two proteins is important for efficient fusion. The fusion activity of Sendai virus has been investigated in terms of its ability to mediate cell-cell fusion [2,4,6], and to fuse with cultured cells

Abbreviations: PS, phosphatidylserine; PG, phosphatidylglycerol; CL, cardiolipin; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; GD_{1a}, disialoganglioside; R-18, octadecylrhodamine.

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[3,7–9], erythrocytes, erythrocyte ghosts and liposomes [3,9–20].

Fluorescence assays for the fusion of lipid enveloped viruses with target membranes have provided extensive information on the kinetics and mechanisms of the interaction of viruses with target membrane components, and of the fusion reaction itself [7,15–30]. In most of these studies, the relief of self-quenching of octadecylrhodamine (R-18) incorporated in the viral membrane, has been used as a reliable measure of virus fusion with target membranes [3,7,15,30]. Although this assay has been utilized extensively in studying the fusion of Sendai virus with liposomes or erythrocyte ghosts, its use in monitoring the kinetics of fusion with cultured mammalian cells has been limited [3,7]. Our recent work with influenza virus has indicated substantial differences in fusion activity toward erythrocyte ghosts, liposomes and cultured cells, particularly in the phenomenon of low pH-induced inactivation of the virus [25,30]. We have therefore studied the fusion of Sendai virus with suspension cells, the human promyelocytic leukemia cell line HL-60 and the human T lymphoblastic leukemia cell line CEM. These cells were chosen for their convenience in monitoring continuously the fluorescence of the virus-cell suspension in a fluorometer cuvette. Fusion was monitored by employing the R-18 fusion assay, and the results were analyzed by means of a mass action model. This model allows for the separate determination of the rate constants of fusion and adhesion, as deduced from the overall fluorescence increase. Previous studies on the fusion of Sendai virus with erythrocyte ghosts [19,20,23] yielded large adhesion rate constants, which means that the potential energy barrier for close approach of the virus to those target membranes is small. As we will show, the emerging rate constants of adhesion of the virus to suspension cells are close to the limit of diffusion-controlled processes. The emerging values of the fusion rate constants for one of the isolates used in this study are, in general, within the range of those previously obtained for virus-ghost and virus-liposome fusion, while for another isolate these values are smaller.

The rate of fusion of Sendai virus with erythrocyte ghosts and mouse spleen cells was found to be optimal at neutral pH [3,16]. Sendai virus-induced cell-cell fusion also occurs at lower or higher pH values, which has led to the suggestion that the overall fusion process of Sendai virus with cells is pH-independent [4]. Detailed studies on the fusion of Sendai virus with liposomes of several compositions, i.e. PS, PG, CL and CL/DOPC [17,20,26], showed enhanced rates and final extents at lower pH (4–5). With DOPC/DOPE/cholesterol/G_{D1a} liposomes [17], however, the rate of fusion was considerably slower than in the former cases, and had a local optimum at pH 7; again, the rate of fusion increased dramatically below pH 5. We have examined several parameters, such as pH and temperature, that may affect the fusion of Sendai virus with the plasma membrane of suspension cells. We have also investigated whether incubation of the virus alone at low pH results in an inactivation of its fusion capacity towards suspension cells, similar to the case with influenza virus [28,31,32]. A certain degree of such inactivation was previously reported [6,16,26,33]. A preliminary account of our observations has been presented [25].

Parts of this work were presented at the American Chemical Society Symposium on Cell and Vesicle Membrane Interactions, Boston, MA (April, 1990), and at the 20th Meeting of the Federation of European Biochemical Societies, Budapest (August, 1990).

Materials and Methods

Cells. The cell lines were obtained from the cell culture facility at the University of California, San Francisco, and were maintained in RPMI 1640 medium containing 25 mM Hepes buffer, supplemented with 10% fetal bovine serum. The cells were grown under a 3% CO_2 atmosphere in a Napco incubator in T-75 flasks, up to a cell density of about 10^6/ml. The cells were harvested by centrifugation in a Beckman TJ-6 cell centrifuge at 1000 rpm for 10 min at room temperature, washed twice in Dulbecco's phosphate-buffered saline, once in Phenol red-free RPMI 1640 containing 25 mM Hepes buffer and resuspended in the latter buffer at a stock cell density of 10^3/ml. They were kept in ice in polypropylene centrifuge tubes until they were transferred to fluorometer cuvettes or other polypropylene tubes. The cells were counted in a hemocytometer, and their viability was determined by Trypan blue exclusion, the percentage of viable cells being routinely between 95 and 98%.

Erythrocyte ghosts were prepared by first isolating the red blood cell fraction from whole blood by centrifugation on Ficoll-Hypaque, washing in phosphate-buffered saline, and then lysing the cells in ice-cold 5 mM EDTA, 10 mM Tes buffer (pH 7.4). The cells were washed repeatedly in this medium, until a colorless preparation was obtained. The ghosts were then resealed by suspension in phosphate-buffered saline containing 5 mM $MgCl_2$, at 37 °C, and allowed to equilibrate for 1 h. They were then pelleted, and resuspended in the RPMI medium without Phenol red.

Virus. The Z/G isolate of Sendai virus Z strain was prepared at the University of Groningen, The Netherlands. It was grown for 72 h in the allantoic cavity of 10-day-old embryonated eggs, purified by differential centrifugation [34], and stored at −70 °C in phosphate-buffered saline. The Z/SF isolate of Sendai virus Z strain, was prepared at the University of Cali-

fornia, San Francisco. It was grown for 48 h at 35.5 °C in the allantoic cavity of 11-day-old embryonated eggs, followed by placing the eggs at 4 °C overnight. The virus was purified from the allantoic fluid collected, by first centrifuging for 30 min at 5000 rpm in a Sorvall RC2-B centrifuge at 4 °C and then at 17000 rpm for 160 min at 4 °C in a Ti-19 fixed-angle rotor in a Beckman L8-M centrifuge. The pelleted virus was resuspended in phosphate-buffered saline and stored at 4 °C overnight. The virus suspension was homogenized and purified by sucrose density gradient centrifugation at 34000 rpm in a Beckman L3–50 ultracentrifuge at 4 °C for 45 min and the collected virus again centrifuged for 45 min at 4 °C at 34000 rpm. The pelleted virus was finally resuspended in Tes-buffered saline and the protein concentration was determined by the Lowry assay. The virus was then stored at −80 °C.

Labeling of virus with R-18. Both virus isolates (Z/SF) and (Z/G) were labeled with R-18 (Molecular Probes, Eugene, OR), by injecting under vortex mixing, an aliquot (10 μl) of a stock R-18 solution to 1 ml of a virus suspension and incubating at room temperature (20 °C) for 0.5–1 h in the dark. The final ethanol concentration was 1%. The amount of the probe added was 20 nmol per 1 mg viral protein, which corresponds to 5 mol% of total viral lipid, assuming that 1 mg viral protein corresponds to 400 nmol of lipid. The unincorporated probe molecules were removed by chromatography on Sephadex G-75 (Pharmacia, Piscataway, NJ) with Tes-buffered saline [15].

Association of Sendai virus with cells. R-18 labeled virus was incubated with cells at 37 °C for 15 min in polypropylene centrifuge tubes. The associated virus and non-associated virus were separated by centrifugation in a Beckman TJ-6 cell centrifuge at room temperature for 10 min at 1000 rpm. R-18 fluorescence in the supernatant (non-associated virus) and in the pellet was determined after addition of 1.25 mM (final concentration) of a polyoxyethylene detergent ($C_{12}E_8$, Calbiochem, San Diego, CA; or $C_{13}E_9$, a gift of Dr. Barry Lentz).

Enzymatic treatment. The virus (10 μg of protein) was pre-incubated with proteinase K (6 μg; Calbiochem) in a final volume of 106 μl at pH 7.5 and 37 °C for 20 min. Subsequently, the pre-treated virus was added to the fluorometer cuvette containing the cells at 37 °C.

Fluorescence measurements. The initial fluorescence of the virus and cell suspension was set to 0% fluorescence. The maximal (100%) fluorescence (F_{max}) was determined by lysing the virus and cell membranes with $C_{12}E_8$ or $C_{13}E_9$ at a final concentration of 1.25 mM and waiting for the fluorescence to equilibrate (5–10 min). Fluorescence measurements were performed in a Spex Fluorolog 2 fluorometer, using the front-face configuration in the emission channel, because of the high concentration of cells employed. High concentrations of cells were chosen to provide maximal binding and fusion sites for a particular concentration of virus that provided ample fluorescence signal. The results presented in Fig. 6 reveal the importance of cell concentration. The excitation was at 560 and the emission monochromator was set at 590 nm (using 5 and 2.5 mm slits in both monochromators), with a high-pass filter between the cuvette and the monochromator, to minimize light scattering (Schott Glass OG590; 50% transmission at 590 nm; Melles-Griot, Irvine, CA).

Analysis of data. The analysis was essentially as described by Nir et al. [19]. In most cases a significant cut in computation times could be achieved by employing the mathematical expressions in Ref. 35. We have employed three parameters: C (M^{-1} 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 experiments were designed to avoid an excessive ratio of virus particles per cell, since previous studies showed that only about 100 particles could fuse with a single erythrocyte ghost [19,36]. Typically 10 μg of viral protein per 2 ml was employed, correponding to $7.3 \cdot 10^{-12}$ M of particles [19]. The concentration of cells in most experiments was $4 \cdot 10^7$ cells/2 ml, which corresponds to $3.3 \cdot 10^{-14}$ M, yielding about 220 virions per cell. The outcome of the measurements of the fraction of virus associated with the cells [36] provided another test for the model calculations and reduced the uncertainty in the values of the parameters C, f and D [19,23,35]. The procedure of pre-binding 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 [23].

Results and Discussion

Fusion of Sendai virus with cultured suspension cells

The fusogenic activity of Sendai virus was investigated using cultured suspension cell lines as target membranes. Octadecylrhodamine (R-18)-labeled virus was incubated with cells either at neutral pH or low pH. Fusion was monitored as the increase of R-18 fluorescence, which results from dequenching of the probe upon dilution into the target membrane [7,15,21]. Fig. 1 shows the time course of R-18 fluorescence during incubation of 5 μg/ml Sendai virus (Z/SF) with $2 \cdot 10^7$ HL-60 cells/ml. The virus fusion activity was lower when the virus was added to cells at pH 5 (curve B). However, when the pH was dropped from 7.5 to 5 after 45 s of incubation (curve C), a very steep increase in fluorescence over a few seconds, followed by a smaller slope, comparable to that seen at pH 7.5 (curve A), was observed. Similar results were obtained

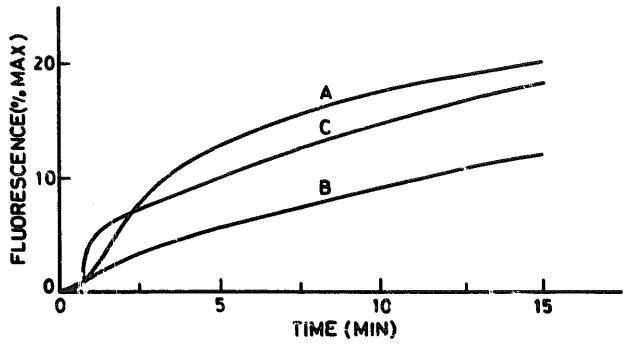

Fig. 1. The time course of R-18 fluorescence dequenching during incubation at 37°C of 5 μg viral protein/ml Sendai virus (Z/SF) with $2 \cdot 10^7$/ml HL-60 cells. (A) pH 7.5, (B) pH 5, (C) pH was lowered to 5 at $t = 45$ s. The value of fluorescence is given as the percent of maximum, and is defined by $F(\%) = 100[I(t) - I_0]/[I(\infty) - I_0]$, where I_0 is the fluorescence intensity at the beginning of the pre-incubation and $I(\infty)$ is the fluorescence at infinite dilution of the probe, achieved by the addition of detergent.

for fusion of Sendai virus (Z/SF) with CEM cells (data not shown).

The possibility of a light-scattering artifact upon lowering the pH was ruled out by the observations that (i) the low pH-induced increase in scattering from a cell suspension without virus, as detected in the emission monochromator, was too small to account for the increase in fluorescence in the presence of virus; and (ii) the initial level of fluorescence when R-18-labeled virus was added to cells at either neutral or low pH was identical.

The results in Fig. 2, obtained with another isolate (Z/G) and either HL-60 or CEM cells, also indicated an enhancement of fusion activity upon lowering the pH (curves A and B). Although no fusion was observed for HL-60 cells at pH 8.95, lowering the pH to 6 induced some fusion (curve C). In contrast to isolate Z/SF, this isolate fused more rapidly and extensively with HL-60 cells, when the pH of the medium was lowered before the addition of virus (see Fig. 3). Since

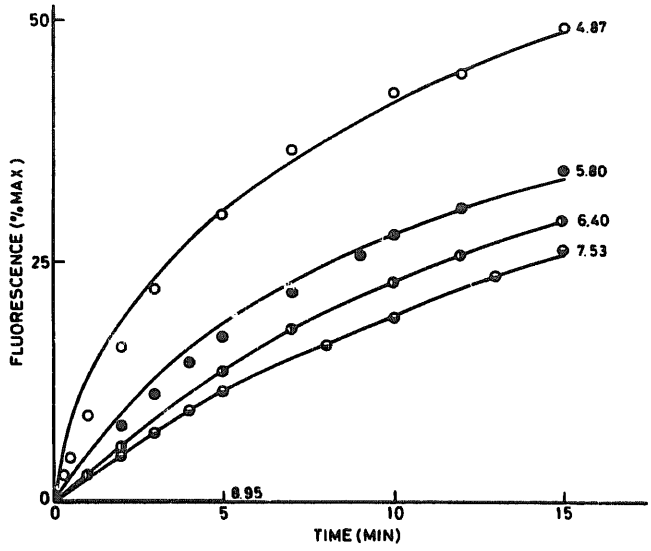

Fig. 3. The effect of pH on the kinetics of fusion of Sendai virus (Z/G; 5 μg viral protein/ml) with $2 \cdot 10^7$/ml HL-60 cells. The virus was incubated with the cells at 37 °C at the indicated pH values. The fluorescence scale was set as in Fig. 1. The symbols indicate calculated values with parameters listed in Table I. Open circles, pH 4.87; closed circles, pH 5.8; right-filled circles, pH 6.40; bottom-filled circles, pH 7.53.

the optimal pH for the fusion activity of Sendai virus towards erythrocyte ghosts is around 7 [16], the fusion of the Z/G isolate with erythrocyte ghosts was monitored. Adding the virus to the ghosts at pH 5 resulted in a reduced rate and extent of fusion compared to adding it at pH 7.5 (data not shown). A previous study on the fusion of Sendai virus with hepatoma cells showed a reduced extent of R-18 dequenching at low pH [7], consistent with our observations with the Z/SF isolate, but differing from that with the Z/G isolate.

The effect of ammonium chloride

Although the Z/SF isolate fused more slowly with suspension cells when the pH of the medium was mildly acidic, the observation that lowering the pH

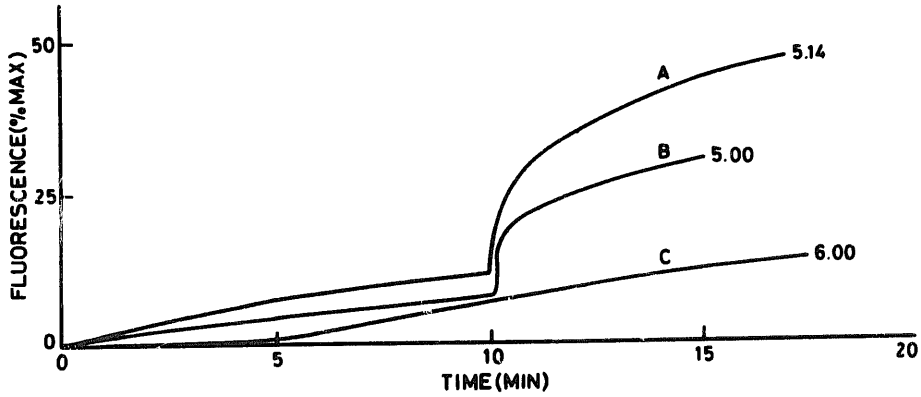

Fig. 2. The time course of R-18 fluorescence dequenching during incubation of 5 μg viral protein/ml Sendai virus (Z/G) with $2 \cdot 10^7$/ml HL-60 (A) and (C) and CEM cells (B). The virus was incubated with the cells at pH 7.5 and 37 °C for 10 min (A) and (B) or at pH 8.95 and 37 °C for 5 min (C) and the pH was lowered to the indicated values. The fluorescence scale was set as in Fig. 1.

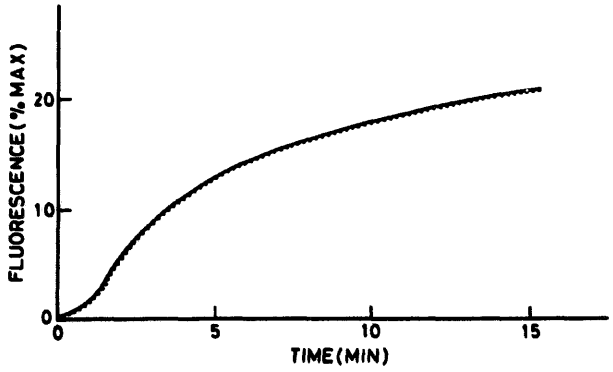

Fig. 4. The effect of NH_4Cl on the R-18 fluorescence increase observed with Sendai virus (Z/SF; 5 μg viral protein/ml) incubated with $2 \cdot 10^7$/ml HL-60 cells at pH 7.5 and 37 °C. The cells were pre-incubated with 20 mM NH_4Cl for 30 min and then incubated with Sendai virus in the presence of the same concentration of NH_4Cl. Solid line, control cells; dotted line, NH_4Cl pre-treated cells. The dashed and dotted lines are superimposable, although in the figure they are separated for clarity. The fluorescence scale was set as in Fig. 1.

after pre-incubation at neutral pH enhanced the fusion, raised the possibility that Sendai virus could fuse with an endosome following endocytosis and acidification of the endosome lumen. The effect of NH_4Cl treatment of cells on virus-cell membrane fusion was therefore investigated, since this agent prevents the acidification of endosomes and lysosomes [29,37,38]. Cells were incubated with 20 mM NH_4Cl [29,38] for 15–30 minutes, and the virus was then added to the medium. Fig. 4 shows that there was no difference in the fusion activity of the virus in the presence and absence of NH_4Cl. Experiments monitoring fusion for 30 min also did not reveal significant differences (data not shown). Although the Z/G isolate had enhanced fusion activity even when added to cells at low pH, its fusion with HL-60 cells was not affected by NH_4Cl treatment (data not shown). The fact that fusion with cells was not inhibited by the presence of NH_4Cl, suggests that, if the virus is internalized in endosomes, the mildly acidic pH of these organelles did not enhance the fusion of the virus with the endosome membrane.

The effect of temperature

Fig. 5 shows the effect of temperature on the fusion of Sendai virus (Z/G) with HL-60 cells. Reduction of the incubation temperature to 20 °C decreased the initial rate and extent of fusion. Fig. 5 also demonstrates a very steep increase in fluorescence over a few seconds followed by a smaller slope when the pH was lowered from 7.6 to 5.0, after 10 min of incubation at 20 °C. It is expected that at 20 °C considerable binding of the virus to the cells occurs, even more extensive than at 37 °C [36], but little fusion occurs at 20 °C and pH 7.6. The steep increase of fluorescence upon lowering the pH most likely reflects the fusion of the pre-bound virus, whose capacity to fuse is increased at low pH.

The temperature dependence of the overall rate of fusion of Sendai virus with suspension cells, is similar to that of the fusion of the virus with liposomes [17] and erythrocyte ghosts [16,23,39]. This effect of temperature may be due to changes in the viral glycoproteins. Lowering the temperature (to 20 °C and below) most likely results in immobilization of viral glycoproteins [23], presumably due to their clustering in the cold, which in turn inhibits the penetration of the N-terminal of the F_1 segment of the viral F glycoprotein into the target membrane [40]. Measurements of the rotational diffusion of Sendai virus envelope proteins showed a decrease when the temperature was reduced from 37 to 22 °C [41]. Previous results on the fusion of phospholipid vesicles with each other [42–44] indicate that the increase in the kinetics with temperature is qualitatively similar to that found in our study. Hence, it is likely that the temperature dependence of the rotational mobility of the viral glycoproteins is

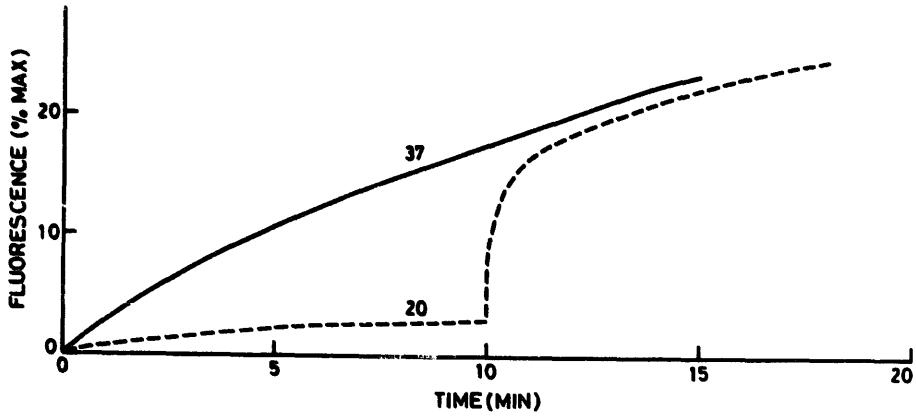

Fig. 5. The effect of temperature on the fusion of Sendai virus (Z/G; 5 μg viral protein/ml) with $2 \cdot 10^7$/ml HL-60 cells. The virus was incubated at pH 7.6 at the indicated temperatures. At 20 °C the pH was lowered to 5.0 after a pre-incubation of the virus with the cells at pH 7.6 for 10 min. The fluorescence scale was set as in Fig. 1.

partly due to the properties of the lipid matrix. In addition, the increase in temperature may also promote significantly the tendency of the lipid molecules in the target membrane to fuse, due to an increase in target membrane fluidity.

The effect of cell concentration

Fig. 6 illustrates the effect of the cell concentration on fusion of the Z/SF isolate with HL-60 cells at neutral pH. Both the rate and extent of fusion were enhanced when the concentration of cells increased from $0.5 \cdot 10^7$ cells/ml to $2 \cdot 10^7$ cells/ml. This observation reflects the fact that at the higher cell concentration, the percentage of the virus bound to the cells is more extensive than that at the lower cell concentration. Thus, the percentage of virus that has fused at each time point (indicated as the percentage change in fluorescence) is higher at the higher cell concentration.

Fusion vs. exchange

Although it has been established before that the increase in fluorescence of the R-18 probe, which is due to its dilution into the target membrane, occurred via fusion of Sendai virus with erythrocyte ghosts or liposomes [15–17,19,20], it was important to re-examine this question for Sendai virus fusion with suspension cells.

Enzymatic treatment of the virus with proteinase K and subsequent addition to HL-60 cells resulted in an increase in R-18 fluorescence of merely 3.5% F_{max} within 15 min (Fig. 7, curve C), compared with about 25% obtained in control experiments (Fig. 7, curve A). Under these conditions, virus binding was reduced from 77.6% (of the added virus) in the controls to 10.1% following enzymatic treatment. The presence of proteinase K, at a concentration equal to that obtained when the pre-treated virus was diluted into the cell suspension (3 μg ml) reduced the final extent of fusion at 15 min to 57% (Fig. 7, curve B) of the value

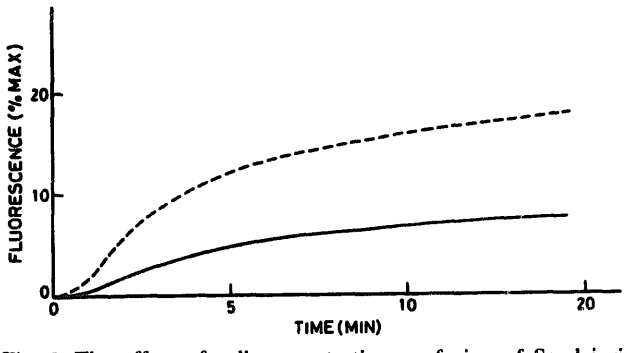

Fig. 6. The effect of cell concentration on fusion of Sendai virus (Z/SF; 5 μg viral protein/ml) with HL-60 cells. The virus was incubated at neutral pH and 37 °C with $2 \cdot 10^7$ cells/ml (dashed line) or $0.5 \cdot 10^7$ cells/ml (solid line). The fluorescence scale was set as in Fig. 1.

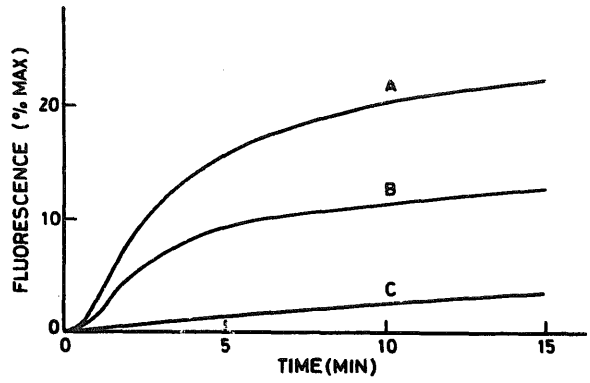

Fig. 7. The effect of pre-incubation of Sendai virus (Z/SF) with proteinase K on its fusion with $2 \cdot 10^7$/ml HL-60 cells. The virus (10 μg viral protein) was incubated with 6 μg of proteinase K at 37 °C and pH 7.5 for 20 min and then incubated with cells at pH 7.5 (curve C). Curves A and B represent the time course of fusion in control experiments (untreated virus), respectively, in the absence and in the presence of proteinase K at a concentration of 3 μg/ml. The fluorescence scale was set as in Fig. 1.

obtained with untreated controls; this is a reduction of 43%. The presence of this very low concentration of the enzyme also reduced the binding of the virus to HL-60 cells from 77.6% (control) to 62.8% of the added virus; this, however, reflects a reduction of only about 19% of the control value. Therefore, despite the retention of 81% of the binding, only 57% of the fusion activity remained. This observation suggests that when the virus is inactivated by enzymatic treatment, the R-18 in the bound virions does not transfer to the target membrane in the absence of fusion. If it had transferred, the fluorescence dequenching in the case of the treated virus would have merely reflected the 19% reduction in binding to the cells. An earlier study by Citovsky et al. [45] showed that trypsin treatment of Sendai virus inhibited R-18 fluorescence dequenching by 91%, when the virus was added to hepatoma tissue culture cells.

To examine the effect of having membranes available for the possible transfer of R-18 in the absence of fusion, we used unlabeled virions. Kim and Okada [46] have reported that 'young' virions are resistant to fusion with each other, even in the presence of poly(ethylene glycol), while aged virions undergo fusion even in the absence of this agent. We examined the effect of having a 19-fold excess of unlabeled virus, in addition to the labeled virus, in the presence of HL-60 cells. In comparison with the control (labeled virus only), the extent of fluorescence increase after a 15 min incubation (at 37 °C, pH 7.5) dropped from 25.1% to 7.9% F_{max} (Fig. 8); i.e. to about 31% of controls, or a reduction of about 69%. The percentages of labeled virus associated with the cells after 15 min (at 37 °C, pH 7.5) were 77.6% and 31.3%, in the absence and in the presence of excess unlabeled virus, respectively; a reduction of 60% when excess virus was present.

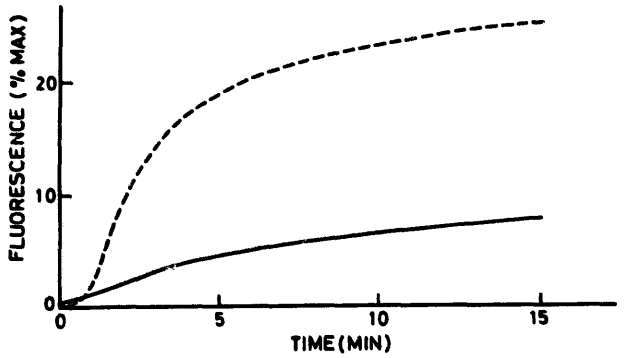

Fig. 8. The time course of -fusion of labeled Sendai virus (Z/SF; 5 μg viral protein/ml), in the presence of a 19-fold excess of unlabeled virus, with $2 \cdot 10^7$/ml HL-60 cells at pH 7.5 and 37 °C (solid line). The dashed line represents the time course of fusion in a control experiment (labeled virus only). The fluorescence scale was set as in Fig. 1.

It can be expected that the exchange of R-18 without viral fusion can only be promoted in the presence of the additional membrane of the unlabeled virus. If it is assumed that all the fluorescence increase observed in the presence of excess unlabeled virus is merely due to exchange and no fusion, an upper bound on the percent of probe exchange during 15 min is about 31%. However, it should be noted that this is not necessarily evidence for probe exchange, but rather an upper bound indicating that most of the observed fluorescence increase is due to the fusion of Sendai virus with the plasma membrane of the suspension cells studied here. Comparison of the percent of virions bound to the cells with the percent of virions undergoing fusion (i.e. % F_{max}), indicates that about 32% (25.1/77.6) of the bound virions have undergone fusion in the control case, while 25% (7.9/31.3) have fused in the case of the excess unlabeled virus. Although this is a small difference, it may imply that, because the unlabeled virions have occupied most of the binding sites, the number of sites the labeled virions can fuse with has been reduced. Thus, not all binding sites for Sendai virus are fusion sites.

Viral inactivation

Pre-incubation of Sendai virus (Z/SF isolate) alone at pH 5 for 15–30 min and 37 °C did not diminish the rate of fluorescence increase (results not shown). This result is in contrast to a certain degree of inactivation of fusion capacity of Sendai virus towards liposomes and erythrocyte ghosts [6,16,26,33]. Pre-incubation of the virus at pH 9 for 15 min at 37 °C did not alter significantly the kinetics of fusion with HL-60 cells at neutral pH. Previously, Hsu et al. [6] had reported that exposure of the virus to basic pH enhanced the fusion activity measured by hemolysis and induction of cell-cell fusion. Whether the differing results are due to the virus isolate or the method of detecting fusion activity (e.g. hemolysis, cell-cell fusion), remains to be investigated. In this regard, Aroeti and Henis [39] have found different molecular requirements for virus-cell fusion and cell-cell fusion. Hoekstra and Kok [3] have reported that pre-incubation of Sendai virus (Z/G isolate) at pH 5–6, or pH 9, reduces extensively the initial rate of fusion of the virus with mouse spleen cells at pH 7.4. Further studies are necessary to examine the reasons for these differing results.

Extensive inactivation of the fusion capacity of influenza virus towards liposomes and erythrocyte ghosts occurs upon incubating the virus alone at pH 5 [28,30–32]. However, we have observed only about 40% inactivation for influenza virus fusing with HL-60 cells [25,30]. This comparison suggests that plasma membranes of suspension cells include certain components whose action counteracts low pH inactivation of both influenza and Sendai viruses. It will be of interest to examine, by sequential removal, which cell surface components might be involved in this process.

TABLE I

Kinetics of fusion of Sendai virus with HL-60 cells at 37 °C: Rate constants of fusion (f) and adhesion (C)

The uncertainty in the parameters C and f (given in squigly brackets) for the isolate Z/G was defined by the range of values beyond which a doubling of the root square mean error resulted. For this isolate the calculated values of fluorescence increase yield good simulation to the experimental values (Fig. 3). For this isolate the calculated fraction of virus associated with the cells after 15 min was 0.3, slightly above the experimental value. The value of D used for this isolate was 0.003 (0.002–0.02) s^{-1}, whereas the range for the Z/SF isolate was (0–0.003) s^{-1} The Z/SF isolate yielded ≥ 0.7 for the fraction of virus associated with the cells; the upper bound on the value of C for the Z/SF isolate was chosen to satisfy this fraction.

Virus isolate and pH	f (s^{-1})	C (10^{10} M^{-1} s^{-1})
Z/G		
7.6	0.1 {0.05–2}	1.2 {(0.7–1.3)}
7.53	0.1 {0.05–2}	1.4 {(1.3–1.5)}
6.4	0.2 {0.1–0.3}	1.7 {(1.5–1.9)}
5.8	0.5 {0.2–1}	2.2 {2–2.4}
4.87	1 {0.2– ≥ 1}	5.2 {5–6}
Z/SF		
7.5	0.008 {0.001–0.01}	2 {1.5–4.5}

Mass action analysis

Table I gives the rate constants that yielded the best fit to the experimental values of fluorescence intensity increase. The calculated values gave very good simulations to the experimental results (see Fig. 3) of fluorescence intensity increase and also agreed with the fraction of virus (isolate Z/G) that was measured to be associated with the HL-60 cells. An attempt to fit similar data for the Z/SF isolate was less successful.

The latter isolate yielded (at neutral pH) similar values or up to 40% less fluorescence increase, but three to 4-fold more virus association with HL-60 cells than the Z/G isolate. This observation indicates that a large fraction of the bound virus was unable to fuse with the cell membrane. One of the reasons for our inability to fit the data may be that this isolate did not exhibit complete (i.e. 100%) fusion activity. The use of a higher rate constant of adhesion (Table I) was essential for explaining the greater association of the Z/SF strain with HL-60 cells. We have also examined whether the data could be fit better by assuming that the Z/SF isolate exhibits a lag time in fusion activity following binding, rather than invoking a low rate constant of fusion. While such an assumption cannot be ruled out completely, its employment did not improve the fit to the experimental values. The lower rate constant of fusion for the Z/SF isolate may be explained by the fact that while a larger fraction of the virus was bound to the cells (compared to Z/G), the extent of fusion achieved was essentially the same as that for the Z/G isolate. The fusion rate constant, f, is derived from the equation $I(t) = [1 - \exp(-ft)]B$, in which $I(t)$ is the normalized fluorescence increase, and B is the fraction of bound virus. Thus, for an identical value of I achieved within a time period t, an increase in B will lead to a lower value of f. Clearly, f reflects an average process for the whole population. It is possible, however, that for the Z/SF isolate a large fraction of the bound virus is unable to fuse at all due to binding to sites unfavorable for fusion, while the other fraction fuses with a higher rate constant than the average f value. In contrast, it is possible that all the virions of the Z/G isolate that bind to the cells bind to favorable fusion sites, therefore exhibiting a complete (100%) fusion activity.

The values deduced for the adhesion rate constant (C), at neutral pH, $(1-4) \cdot 10^{10}$ M^{-1} s^{-1} are several-fold larger than those obtained for Sendai virus interacting with erythrocyte ghosts ($(1-4) \cdot 10^{9}$ M^{-1} s^{-1}) [19,23], and two orders of magnitude larger than the values for virus-liposome aggregation ($(7-9) \cdot 10^{7}$ M^{-1} s^{-1}). The upper limit on C-values in diffusion-controlled processes [47] at 37 °C is $5 \cdot 10^{9}$ M^{-1} s^{-1} in the case of aggregation of spherical particles of equal sizes, but C-values can be larger for spherical particles of unequal sizes [48]. Thus, the trend of increase in C-values in the sequence liposomes < erythrocyte ghosts < suspension cells, may largely reflect the increase in particle sizes. However, it is clear that the rate of viral adhesion is close to the diffusion-controlled limit, which means that only a small potential barrier exists for a close approach of the virus to plasma membranes or to those of erythrocyte ghosts.

Within experimental uncertainty, the range of fusion rate constants (at neutral pH) in Table 1 is similar to that found in previous studies for Sendai virus fusing with erythrocyte ghosts and liposomes [19,20,23]. However, the liposomes used for these studies were composed of acidic phospholipids (CL, CL/DOPC and PS). Significantly smaller fusion rates are obtained when liposomes are enriched with PC [17]. Hence, at this stage, we can only deduce that the sum of effects of non-lipid components in cellular plasma membranes does not result in an inhibition of the fusion of Sendai virus with suspension cells. It is possible that the plasma membrane promotes the fusion reaction to a certain degree. The effect of lowering the pH on enhancing the fusion capacity of Sendai virus towards suspension cells remains to be understood in molecular terms. A similar effect was observed in the case of acidic liposomes, such as CL, CL/DOPC and PS [17,20], but not in the case of erythrocyte ghosts [16], or DOPC/DOPE/cholesterol/GD_{1a} (7:3:6.6:1.1) liposomes [17].

Conclusion

Our results indicate that the kinetics of fusion of Sendai virus with cultured suspension cells depend on the virus isolate, and differ in several respects from fusion with erythrocyte ghosts or liposomes. These differences include the enhancement of the rate and extent of fusion with cells at low pH for one of the isolates, the absence of low pH-mediated inactivation of the virus, and the high adhesion rate constant.

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