Sindbis Virus-mediated Cell Fusion from Without Is a Two-step Event

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SUMMARY

The process by which Sindbis virus induces the fusion of BHK-21 cells in monolayer cultures after exposure to low pH was examined. The fusion process was found to require an exposure to low pH followed by a return to conditions closer to neutral pH. The rate at which cells fused was affected by the pH to which the monolayers were exposed, subsequent to the initial exposure to acid conditions. Virus-mediated cell fusion was not observed if the virus–cell complexes were maintained at the acid pH required for membrane fusion.

In a now classic series of investigations, Helenius and co-workers have shown that membrane-enveloped viruses such as the alphaviruses can induce the fusion of cell membranes if virus–cell complexes are exposed to an acidic pH (White et al., 1983). This observation, combined with the observation that compounds that elevate the pH of normally acidic cell compartments (lysosomotropic weak bases) block Semliki Forest virus replication at an early stage (Helenius et al., 1982), has led to the development of a widely accepted model which proposes that viruses achieve entry into cells by a fusion event which occurs in an acidic intracellular compartment (Marsh et al., 1983). (For a review of virus penetration, see Dimmock, 1982.)

The alphaviruses Sindbis and Semliki Forest are among a large group of enveloped viruses which are capable of inducing fusion of cultured cells. Alphavirus-induced cell fusion requires high multiplicities of viruses attached to the surface of the cells and the exposure of the virus–cell complex to acid pH (White et al., 1983; Edwards et al., 1983; Mann et al., 1983). Acid pH has been demonstrated to induce conformational changes in alphavirus envelope proteins and the conformational changes are suspected to expose protein domains which participate in the membrane fusion event (Edwards et al., 1983; Schmaljohn et al., 1983). Other enveloped viruses, such as rhabdoviruses and myxoviruses, have also demonstrated membrane-fusing capability after exposure to acid conditions (see White et al., 1983). The discovery of low pH-induced, virus-mediated membrane fusion has played an important role in the development of a hypothesis which suggests that enveloped viruses penetrate cells by a pathway involving an acidic intracellular compartment (Marsh et al., 1983). This theory suggests that viruses such as the alphaviruses exploit the normal cellular process of receptor-mediated endocytosis to achieve entry into the cell cytoplasm. Viruses attaching to cell surface receptors are drawn into endosomes. The subsequent acidification of the endosome (a normal cellular event essential for the processing of receptor-bound ligands) is believed to induce fusion of the virus membrane with the endosomal membrane, releasing the virus genome into the cytoplasm.

In previous reports in which we examined the process of fusion of tissue-cultured cells by Sindbis virus, we found that Sindbis virus-induced fusion from without required exposure to an acid environment but that membrane fusion itself only occurred after the virus–cell complex was returned to neutral pH conditions (Mann et al., 1983; Edwards & Brown, 1984). Other investigations examining cell fusion by the alphavirus Semliki Forest virus or rhabdo- or myxoviruses also employed transient exposure to low pH, followed by a return to neutral pH conditions, in order to demonstrate virus-mediated cell fusion (White et al., 1980, 1981). Because the pH of the endosome does not fluctuate from acid to neutral pH and because the laboratory
phenomenon of low pH-mediated, virus-induced cell fusion is widely believed to mimic those events which occur as enveloped viruses penetrate a host cell, we have attempted to determine what pH parameters are required to produce fusion of BHK-21 cells by Sindbis virus.

For these studies, we employed BHK-21 cells and a heat-resistant wild-type strain of Sindbis virus (SVHR) with a particle:p.f.u. ratio of between 1:6 and 1:10, as previously described (Mann et al., 1983). Fusion of BHK cells was carried out as described by White et al. (1981), Mann et al. (1983) and Edwards et al. (1983). Monolayers of BHK-21 cells, at a cell density of 1.5 x 10^5/cm², were exposed to Sindbis virus at a m.o.i. of 1000 p.f.u./cell for 1 h at 4 °C to allow attachment of virus in the absence of internalization. The cells were then exposed for 5 min at 37 °C in fusion medium: Eagle's MEM without bicarbonate containing 0-2% foetal calf serum, 10 mM-MES and 10 mM-HEPES, essentially as described by White et al. (1981). The medium was adjusted to the desired pH by the addition of sodium hydroxide. After exposure to a particular pH the cells were, in some cases, returned to similar medium at higher pH values to evaluate the effect of this on cell fusion. Cell fusion was evaluated as described previously (Edwards & Brown, 1984). Stained or unstained, fixed (glutaraldehyde) or unfixed monolayers were examined and photographed by phase-contrast light microscopy and the extent of fusion was determined by counting the number of nuclei and number of cells in a unit area. In making this evaluation, we determined the average number of nuclei within the confines of a recognizable cell membrane (polykaryon).

As demonstrated previously by our laboratory (Mann et al., 1983), we found that a complex of BHK-21 cells and SVHR required exposure to a pH of 5.3, or lower, for maximum fusion to be observed 1 h after returning to pH 7.2 (see Fig. 1). This pH optimum coincides with the pH at which the conformational changes in the virus envelope proteins take place (Edwards et al., 1983). Fig. 1 also shows the effect of shifting the pH of the virus–cell complex to values between pH 5.3 and 7.2 after initial exposure at pH 5.3. We found that maximum fusion of cells was observed 1 h after increasing the pH environment to pH 6.5 or higher. Intermediate degrees of cell fusion were observed after shifting pH 5.3-treated cell cultures to pH values between 5.8 and 6.5. These experiments produce two non-overlapping curves which indicate two optimal pH
values to which Sindbis virus–cell complexes must be sequentially exposed for efficient cell fusion to take place.

The observation that intermediate degrees of cell fusion occurred when virus–cell complexes were brought to pH values between 5.8 and 6.5 (Fig. 1) suggested that fusion at these values occurred at a slower rate. To test this, BHK-21 cell monolayers with attached Sindbis virus were exposed to pH 5.3 for 5 min and then placed in media at pH 5.3, 6.0 or 6.5 for periods of 1, 2 or 3.5 h. At the end of these times, the extent of cell fusion was determined. As shown in Fig. 2, we found that cells maintained at pH 5.3 did not demonstrate measurable levels of fusion in the 3.5 h incubation period. Cells placed at pH 6.5 demonstrated maximum fusion in the first hour. Cells at pH 6.0 achieved maximum fusion in 3.5 h, with intermediate values at 2 h and 1 h. The rate at which Sindbis virus–cell complexes fuse is therefore affected by the pH in which the cells are placed after exposure to pH 5.3.

We conclude that Sindbis virus-mediated fusion of cultured cells is a two-step event, each step demonstrating a different pH requirement. The virus must first be exposed to pH 5.3. Previous studies have shown that this pH induces a conformational change in the virus glycoproteins and that this change is apparently required for membrane fusion to take place (Edwards et al., 1983; Schmaljohn et al., 1983). The fusion event itself, as visualized in these studies, does not occur at pH 5.3 but requires a shift in the pH of the medium to conditions closer to neutrality. The requirement for a shift to higher pH indicated either a second change in the virus particles at that pH or some other requirement placed on the fusion system by the host cell. We have previously demonstrated that both vertebrate and invertebrate cells require different pH values for fusion by the same virus, suggesting that the host cell membrane contributes in some unknown way to the fusion event (Edwards & Brown, 1984).

The observation presented here that Sindbis virus-mediated fusion of BHK-21 cells requires a shift from neutral pH conditions (attachment) to low pH (virus conformational change) to a higher pH (membrane fusion) suggests that this laboratory phenomenon may not duplicate or accurately reflect the events that occur during the normal fusion of a virus membrane with a cell membrane. There is presently no evidence that an endosomal compartment shifts towards acid pH conditions and then subsequently towards neutral pH. Furthermore, we have previously shown that weak bases such as chloroquine and ammonium chloride (which are commonly used to raise the pH of intracellular compartments) do not prevent the expression of Sindbis virus genes responsible for the establishment of homologous interference, suggesting that passage through an acidic intracellular compartment may not be essential for penetration of cells by these viruses (Cassell et al., 1984). It has also been demonstrated by Haywood & Boyer (1985) that influenza virus membranes can fuse with liposomes at pH 7.5 and that the composition of the liposome dictates the pH required to initiate fusion. Haywood & Boyer concluded that virus particles are capable of fusing their membranes with cell membranes at any pH where adequate binding occurred. Acid pH may be required in certain instances to overcome charge repulsion between the virus and cell surface to establish binding. The data presented in this paper support that hypothesis. It is therefore possible that the changes in virus envelope protein conformation and other parameters required for mediating the fusion of cell and viral membranes are established by some as yet unknown interaction of the virus with the host cell membrane and that these conditions are established in the absence of an alteration in the environmental pH.

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REFERENCES


Short communication


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