Articles

Sendai Virus Membrane Fusion: Time Course and Effect of Temperature, pH, Calcium, and Receptor Concentration†

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ABSTRACT: The conditions that optimize Sendai virus membrane fusion with liposomes have been studied. No fusion occurs in the absence of ganglioside receptors. Maximum fusion occurs when the molar ratio of ganglioside G_{D1a} to phospholipid is 0.02 or greater. The amount of fusion at 37 °C increases with time up to at least 6.5 h. The rate of fusion increases from the lowest temperature tested, 10 °C, to 40 °C. Above 43 °C the amount of fusion decreases because of thermal inactivation of the viral proteins. There is a broad pH maximum between pH 7.5 and pH 9.0. At both ends of the pH range the amount of fusion increases and exceeds that found in the physiologic pH range. Neither ethylenediaminetetraacetic acid nor Ca^{2+} changes the amount of membrane fusion. The optimal conditions for membrane fusion of Sendai virus membranes with liposomes are the same as the optimal conditions for fusion with host cells and with red blood cells. Since the liposomes contain no proteins, the optimal conditions for Sendai virus membrane fusion must be determined by the viral proteins and be mostly independent of the nature or presence of the host proteins.

Paramyxovirus membranes are very simple biologic membranes (Lenard, 1978; Compans & Klenk, 1979) which have as their major function adsorption to the host cell followed by entry into the cell. Currently it is thought that entry is mainly by fusion of the viral and host membranes. The lipids of the viral membranes are derived from the plasma membranes of their host cells (Blough & Lawson, 1968; Klenk & Choppin, 1970). The membrane of egg-grown Sendai virus, a paramyxovirus, contains 46% unsaturated fatty acids in its phospholipid fraction and approximately 30% cholesterol (Blough & Lawson, 1968). The three viral membrane proteins are coded for by the viral RNA. These proteins include the two glycoprotein spikes, the HN protein and the F protein, both of which are necessary for membrane fusion. The HN protein has both receptor-binding (hemagglutinating) and sialidase (neuraminidase) activities (Scheid et al., 1972; Nagai & Klenk, 1977). The cleaved form of the F protein is necessary (although not sufficient) for membrane fusion and viral infectivity (Homma & Ohuchi, 1973; Scheid & Choppin, 1974), but its mechanism of action is not known. The F_1 protein N terminus generated by proteolytic cleavage of the precursor F_0 protein is followed by a sequence of 15 uncharged amino acids (Gething et al., 1978). Because oligopeptides containing the same amino acid sequence as this hydrophobic N terminus of the F_1 protein inhibit viral infection and viral-induced fusion (Richardson et al., 1980) and because cleavage of the F_0 protein to form the F_1 protein appears to be accompanied by an increase in the exposed hydrophobic surface, it has been hypothesized that the F protein acts by inserting its hydrophobic moiety into the host bilayer (Hsu et al., 1981). On the other hand, Knutton (1978) showed that the structural changes in the viral surface that are a prerequisite for membrane fusion only occur in the presence of the active F_1 protein and suggested that the function of the F protein is to bring about this reorganization of the viral membrane. The M protein when isolated forms filaments (Hewitt & Nermut, 1977) and is thought to form a shell on the inner side of the bilayer to form a "viroskeleton" (Kim et al., 1979). The M protein is thought to bind the nucleocapsid to the membrane (Shimizu & Ishida, 1975; Yoshida et al., 1976). What role the M protein plays in membrane fusion and whether the internal viral proteins have any part in membrane fusion are not known.

Sendai virus uses gangliosides as receptors when the gangliosides are incorporated into liposomes (Haywood, 1974a), and Sendai virus membranes can fuse with ganglioside-containing liposomes after incubation at 37 °C (Haywood, 1974b). When the steps of Sendai virus membrane fusion with liposomes are followed by microscopy (Haywood & Boyer, 1981), these steps appear to be exactly similar to those of Sendai virus membrane fusion with host cell plasma membranes (Morgan & Howe, 1968). Since the liposomes contain no proteins, have no metabolic activity, and have no ionic or osmotic gradient across their membranes, the fact that Sendai virus membranes can fuse with liposomes strongly suggests that the viral membrane plays the major role in directing the

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fusion process. In this work the time course of Sendai virus membrane fusion with liposomes and the effect of temperature, pH, and calcium are studied. A comparison of these conditions with the conditions that optimize fusion with host cells or with another model system, the red cell, makes it possible to separate what factors in membrane fusion are determined by the viral membrane and what factors are determined by the host membrane.

Materials and Methods

Buffers. NTE contains 50 mM NaCl, 1 mM EDTA,[1] and 10 mM Tris, pH 7.4. PBS contains 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, with the pH adjusted to 7.4 unless stated otherwise. HKN contains 30 mM Hepes [*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid], pH 7.4, 2.7 mM KCl, and 130 mM NaCl. Mes buffer contains 30 mM Mes [2-(*N*-morpholino)ethanesulfonic acid], 2.7 mM KCl, and 130 mM NaCl. Mes–Hepes buffer contains 15 mM Mes, 15 mM Hepes, 2.7 mM KCl, and 130 mM NaCl.

Virus. Virus was grown in 10-day-old embryonated chicken eggs. A fresh inoculum was prepared by infecting a few eggs at low multiplicity (10^{-4}–10^{-3} HAU/egg). The allantoic fluid from this passage was used within a day to infect more eggs at a multiplicity of 1 HAU/egg. After 15-h incubation at 37 °C, 0.1 or 0.2 mCi of [^{35}S]methionine (Amersham) was injected into each egg. After 64–72-h total incubation the allantoic fluid was harvested and clarified by centrifuging twice at 4000*g* for 10 min. The clarified allantoic fluid was layered upon a pad of 60% sucrose in NTE and centrifuged at 26 000 rpm for 1.5 h in an SW28 rotor to concentrate the virus. The virus band was collected, diluted with NTE, layered on a 35–60% (w/v) continuous sucrose gradient, and centrifuged at 20 000 rpm overnight (15.7 h) in an SW27.1 rotor. One-milliliter fractions were collected, and the counts and HAU were measured. The tubes containing the center of the virus peak were combined (2–4 mL) and dialyzed against PBS. The specific activities of the virus preparations used were 7.5 (Figures 1 and 2), 10 (Figure 3), and 21 cpm/HAU (Figure 4). (One HAU contains roughly 10^7 virus particles and 10^6 plaque forming units.)

Lipids. Egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE) were obtained from Avanti Biochemicals, Inc., and cholesterol, chromatography standard grade, was obtained from Sigma Chemical Co. These lipids each gave only one spot when 0.8 μmol was chromatographed with chloroform/methanol/concentrated NH_4OH (60:25:4 v/v/v) and with chloroform/methanol/glacial acetic acid/water (65:25:2:4 v/v/v/v) on silica gel G TLC plates. Ganglioside G_{D1a} was obtained from Supelco, Inc. Only one spot was obtained when 0.3 μmol was chromatographed with chloroform/methanol/2.5 M NH_4OH (60:35:8 v/v/v) plus 20 mg of KCl/100 mL.

Liposomes. Multilamellar liposomes were made as previously described (Haywood & Boyer, 1981). The liposomes were made of 0.7 μmol of PC, 0.3 μmol of PE, 0.66 μmol of cholesterol, and 0.03 μmol of ganglioside G_{D1a} except in those experiments where the ganglioside content was varied. The dried lipids were resuspended in 0.15 mL of buffer at room temperature (22–24 °C).

[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; PBS, phosphate-buffered saline; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*'-2-ethanesulfonic acid; HAU, hemagglutinating unit.

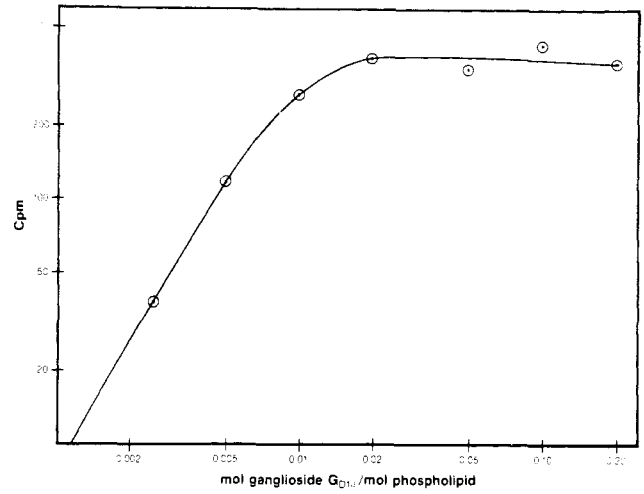

FIGURE 1: Dependence of Sendai virus membrane fusion upon liposomal G_{D1a} concentration. Liposomes were made in PBS from 0.7 μmol of PC, 0.3 μmol of PE, 0.66 μmol of cholesterol, and different amounts of ganglioside G_{D1a}. ^{35}S-Labeled virus was adsorbed at 0 °C and incubated for 2 h at 37 °C. The amount of ^{35}S-labeled viral protein incorporated into liposomes was counted.

Fusion of Sendai Virus Membranes with Liposomes. ^{35}S-Labeled virus (5000 cpm) was adsorbed to liposomes in 0.3 mL of buffer at 0 °C for 1 h and then incubated at 37 °C for 2 h unless stated otherwise. The mixture was then returned to the ice bath. Fusion of the viral membrane with the liposome results in incorporation of the viral membrane proteins into the liposomal membrane and deposition of the viral internal proteins inside the liposome. For removal of the viruses that have adsorbed to the liposome but have not fused, 60% sucrose in NTE was added to the liposome–virus mixture to bring it to a final concentration of 40% sucrose. This was layered over 0.3 mL of 60% sucrose and under 3.3 mL of 30% sucrose and 0.5 mL of PBS. In a few experiments the virus–liposome mixture was brought to a final concentration of 30% sucrose and layered under 3.6 mL of 20% sucrose and 0.5 mL of NTE. This discontinuous gradient was then centrifuged at 45 000 rpm for 45 min at 4 °C in an SW65 rotor. Both the fused and the unfused liposomes rise to the sucrose–buffer interface while the viruses are forced down onto the 60% sucrose. One-milliliter fractions were collected and counted. Under conditions where there is no membrane fusion such as when F_0 virus is used (A. M. Haywood, unpublished results) or when microscopy shows no fusion has occurred as is the case if the virus and liposomes are kept at 0 °C, 2% of the counts in the virus initially added remain with the liposome. This 2% background was subtracted from the total counts incorporated into liposomes. The quantitative estimate of the amount of membrane fusion as determined by the amount of labeled viral protein incorporated into liposomes always agrees with the more qualitative estimate obtained by electron microscopy.

Results and Discussion

Concentration of Ganglioside G_{D1a} Needed for Membrane Fusion. Previous work (Haywood, 1974b) has shown that Sendai virus fuses well with liposomes that contain PC, PE, cholesterol, and mixed brain gangliosides. The mixed brain gangliosides contain five main species of gangliosides not all of which have receptor activity (Haywood, 1975; Holmgren et al., 1980). In this work the ganglioside G_{D1a}, which has Sendai virus receptor activity (Holmgren et al., 1980), was substituted for mixed brain gangliosides. G_{D1a} was chosen since it is abundant in brain gangliosides and easy to purify.

For determination of the concentration of ganglioside G_{D1a} needed for membrane fusion to occur, liposomes were made containing different amounts of ganglioside G_{D1a}. Samples were taken for each ganglioside concentration after adsorption at 0 °C for 1 h to confirm that unfused virus was removed equally well from liposomes containing different amounts of ganglioside. Figure 1 shows the incorporation of labeled viral proteins into liposomes due to membrane fusion after adsorption at 0 °C and incubation at 37 °C for 2 h. Fusion did not occur when there was no ganglioside in the liposomes. The amount of fusion of Sendai virus membranes with liposomes increased with increasing ganglioside G_{D1a} concentration up to a molar ratio of ganglioside to phospholipid of 0.02. The relationship between ganglioside concentration in the membrane and membrane fusion observed in this study using ganglioside G_{D1a} is similar to the relationship between ganglioside concentration and virus binding observed previously using a mixture of gangliosides (Haywood, 1974a). This is consistent with the fact that virus binding is necessary for membrane fusion to occur as are the structural changes in the membrane which result from this binding (Haywood & Boyer, 1981). These data do not eliminate the possibility that gangliosides also have other roles in membrane fusion.

Landsberger et al. (1981) have shown that when they measure phospholipid transfer from Sendai virus to liposomes, they observe 30% transfer with liposomes that do not contain any gangliosides and then an additional 10–30% transfer with liposomes that do contain gangliosides. One of the major problems in trying to quantitate membrane fusion by phospholipid transfer is that membranes often transfer lipids in the absence of membrane fusion. It has been demonstrated by two different experimental methods that some phospholipids exchange between Sendai virus and red cells without accompanying membrane fusion (Maeda et al., 1977, 1981a). The estimate was made that 30% of the viral phospholipids were exchanged without accompanying membrane fusion (Maeda et al., 1977). The fact that Landsberger et al. (1981) find 30% of the viral phospholipid is transferred to liposomes without gangliosides suggests that the virus can exchange 30% of its phospholipid with the liposome as well as with the red cell without accompanying membrane fusion. If the phospholipid transfer in excess of 30% represents membrane fusion, the data of Landsberger et al. (1981) are consistent with these data obtained by measuring viral protein transfer.

To determine if liposomes containing receptor gangliosides other than G_{D1a} can fuse with virus, liposomes were made that contained PC, cholesterol, PE, and trisialogangliosides from Supelco. These trisialogangliosides contain the receptor ganglioside G_{T1b} and 10–30% of the nonreceptor ganglioside G_{D1b}. Virus fused with these liposomes, so ganglioside G_{T1b} is able to fulfill the receptor ganglioside requirement for fusion.

Supelco gangliosides have been shown by radioiodination to contain a small amount of protein (Markwell et al., 1981), but chicken brain ganglioside G_{D1a} prepared by R. W. Ledeen contained less than 0.02% protein by dry weight detectable by radioiodination (Markwell, personal communication). Dr. R. W. Ledeen kindly gave us some of his bovine brain ganglioside G_{D1a} and chicken brain ganglioside G_{D1a}. Liposomes that contained 3 mol % of either Supelco bovine brain ganglioside G_{D1a}, Ledeen's bovine brain ganglioside G_{D1a}, or Ledeen's chicken brain ganglioside G_{D1a} were incubated with radiolabeled virus for 2 h at 40 °C, and the number of counts in viral protein incorporated into these liposomes was found to be 493, 519, and 516, respectively. Therefore the small amounts of protein in the Supelco gangliosides did not appear to have any bearing upon the ganglioside requirement for membrane fusion.

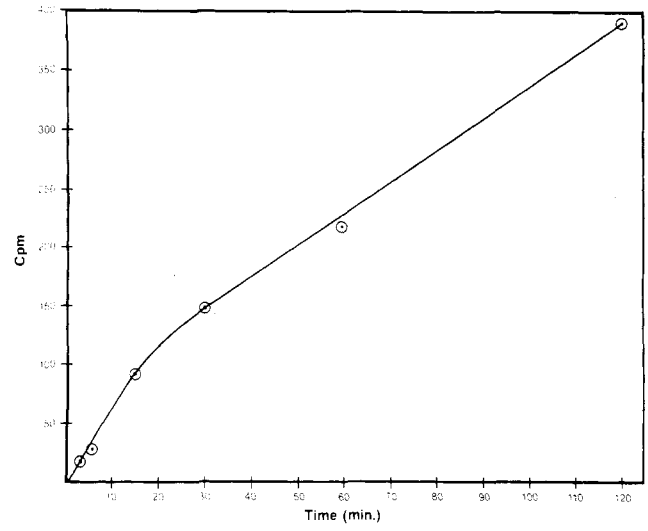

FIGURE 2: Time course of fusion of Sendai virus membranes with liposomes. ^{35}S-Labeled Sendai virus was adsorbed for 1 h at 0 °C to liposomes containing 0.7 μmol of PC, 0.3 μmol of PE, 0.66 μmol of cholesterol, and 0.03 μmol of ganglioside G_{D1a} in PBS and then incubated at 37 °C for different time periods. The amount of ^{35}S-labeled viral protein incorporated into liposomes was counted.

It has long been known that sialic acid containing receptors are necessary for paramyxovirus infection (Hirst, 1959), and it has recently been found that when cellular receptors are removed by sialidase, Sendai virus can enter cells only if the receptor activity of the host membrane is restored by the addition of gangliosides with receptor activity (Markwell et al., 1981). Therefore the requirement for the presence of a receptor for viral entry is similar for liposomes and for host cells.

For the following experiments a molar ratio of ganglioside G_{D1a} to phospholipids of 0.03 was chosen.

Time Course of Membrane Fusion. The amount of fusion that occurred during different times of incubation at 37 °C is shown in Figure 2. Fusion occurs most rapidly during the first 15 min at 37 °C but continues thereafter until 2 h. In other experiments fusion was shown to continue steadily up to at least 6.5 h. After 18-h incubation, 939 cpm, which is 19% of the total cpm in the virus added, were incorporated into liposomes. With a different virus preparation 27% of the total counts added were incorporated into liposomes by 18 h. This was apparently the maximum number of viruses that could fuse because the amount of fusion did not increase with a 24-h incubation at 37 °C or with an 18-h incubation at 43 °C. The time course of the fusion of Sendai virus membranes with liposomes is consistent with the observation that virus-induced cell–cell fusion is extended over several hours (Poste & Pasternak, 1978). It is also generally consistent with the time course observed for fusion of virus with the red cell (Maeda et al., 1981a), although Lyles & Landsberger (1979), using a different procedure, found a half-time of 7 min for mixing of viral and red cell lipids. The steady rise in the amount of fusion over many hours is not as clear with red cells or host cells presumably because other events such as hemolysis and viral replication prevent detection of later fusion.

Temperature Dependence of Membrane Fusion. The amount of membrane fusion that occurs over 2 h at different temperatures was measured (Figure 3). The amount of fusion reached clearly detectable levels as the incubation temperature approached 20 °C and continued to increase with increasing

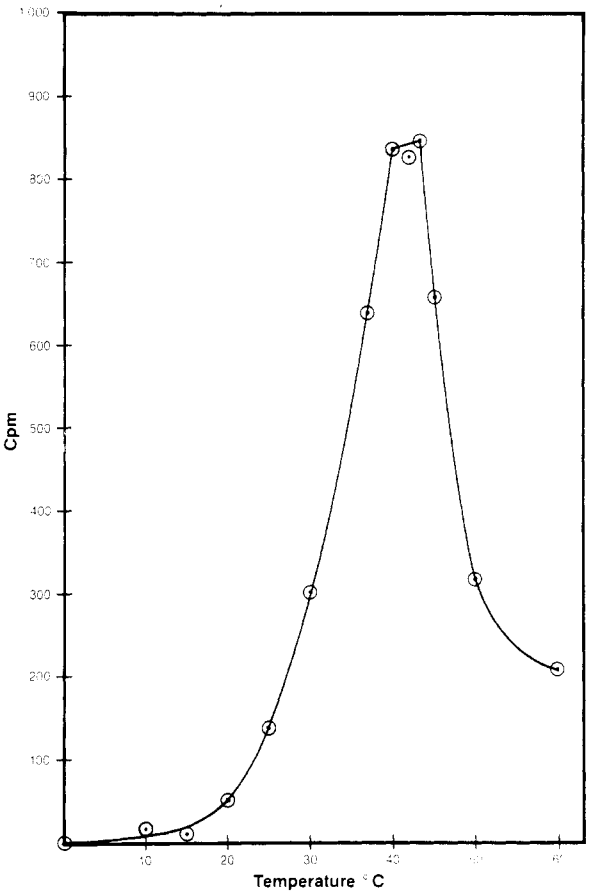

FIGURE 3: Temperature dependence of fusion of Sendai virus membranes with liposomes. ^{35}S-Labeled Sendai virus was adsorbed to liposomes in PBS for 1 h at 0 °C and then incubated at different temperatures for 2 h. The amount of ^{35}S-labeled viral protein incorporated into liposomes was counted.

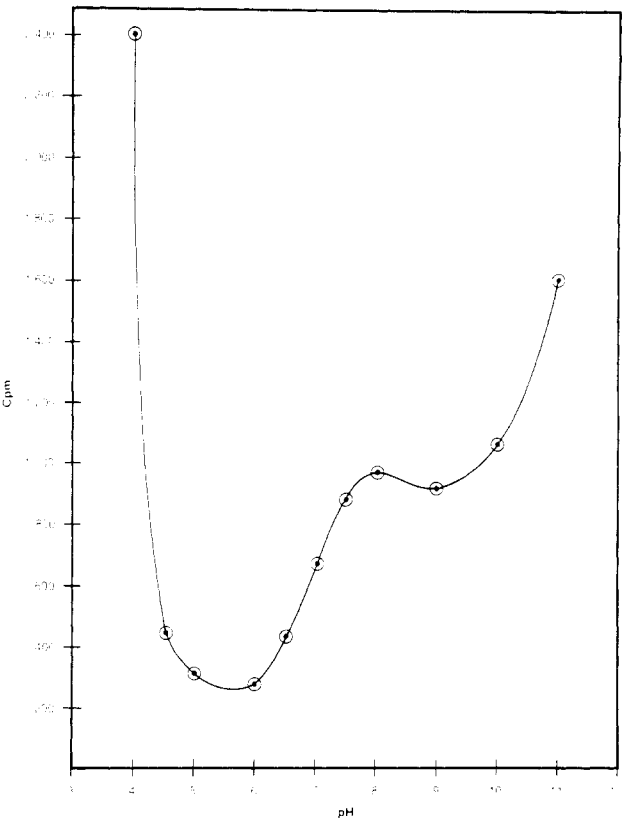

FIGURE 4: Effect of pH upon fusion of Sendai virus membranes with liposomes. Liposomes were made in PBS which had been adjusted with HCl or NaOH to different pHs. ^{35}S-Labeled virus was dialyzed against PBS, pH 7.4, which contained one hundredth the usual amount of phosphate. Addition of virus to liposomes resulted in a 12.5-fold dilution of virus and did not alter the pH of the media containing the liposomes. Virus was adsorbed to the liposomes for 1 h at 0 °C and then incubated at 37 °C for 2 h. The amount of ^{35}S-labeled viral protein incorporated into liposomes was counted.

temperatures up to 40 °C. The amount of fusion decreased at temperatures above 43 °C. Some fusion occurred when samples were incubated at 60 °C, but a comparison of the amounts of fusion occurring after 5 min and after 2 h at 60 °C showed that all the fusion occurred in the first 5 min. Since lipids but not proteins should be expected to tolerate a temperature of 60 °C, the decrease in fusion at higher temperatures is likely to be due to thermal inactivation of the viral proteins. Since the temperature dependence of fusion of Sendai virus with liposomes resembles the temperature dependence of Sendai virus fusion with host cells and with red blood cells, the temperature dependence is probably mainly determined by the properties of the viral membrane and especially the viral membrane proteins. Thus a similar temperature maximum and minimum have been observed for hemolysis by Sendai virus (Neurath, 1965), and 19 °C has been given as a minimum temperature for phospholipid transfer between Sendai virus and red blood cells (Maeda et al., 1975). In their review on virus membrane fusion, Poste & Pasternak (1978) state that the frequency of paramyxovirus membrane fusion drops rapidly with reduction of temperature and is almost completely inhibited below 18 °C. They also suggested that this inhibition of fusion below 18 °C could result from reduced membrane fluidity. Since both the liposomes and the viral membranes contain a high proportion of unsaturated phospholipids and of cholesterol, a major change in the properties of the lipids at 18 °C is not likely. Since the rate of fusion is very temperature dependent, it is possible that the rate of fusion at temperatures below 18 °C makes fusion undetectable over the time intervals usually studied. Because the liposome system has the advantage that fusion can be studied over long periods of time, virus was incubated with liposomes at 10 °C for 24 h. During this period 88 cpm in viral protein was incorporated into liposomes. Therefore, some membrane fusion does occur at 10 °C, but at a very slow rate.

Effects of pH upon Fusion of Sendai Virus Membranes with Liposomes. Figure 4 shows the amount of Sendai virus membrane fusion after 2-h incubation at 37 °C in PBS adjusted to different pHs between 4 and 11. There is a broad maximum in the amount of fusion between pH 7.5 and pH 9.0 and a minimum in the amount of fusion between pH 4.5 and pH 6.5. As the pH goes to both ends of the pH range, the amount of fusion increases above that found at physiologic pHs. Electron microscopy shows that the fusion at pH 11 is qualitatively similar to that at pH 7.5, but that at pH 4 many of the viruses which have not fused have formed very large aggregates. It is possible that the formation of aggregates at pH 4 alters the fusion assay, although a control experiment at pH 4 where the viruses were only adsorbed at 0 °C and not incubated at 37 °C showed no fusion. The pH optimum observed in these experiments is similar to that observed for phospholipid transfer from Sendai virus to erythrocytes (Lyles & Landsberger, 1979). This suggests that the pH optimum for Sendai virus membrane fusion is determined primarily by the viral proteins rather than by the host cell membrane. Sendai virus hemolysis, however, has been reported to have a broader optimum which starts as low as pH 5.5 or 6.0 (Hosaka, 1958; Neurath, 1965; Maeda & Ohnishi, 1980). The differences in the pH optimum of phospholipid transfer into

Table I: Effect of EDTA and Ca^{2+} upon Fusion of Sendai Virus Membranes with Liposomes[a]

buffer	HKN	HKN and 10^{-3} M EDTA	HKN and 10^{-3} M $CaCl_2$
cpm	412	424	399

[a] Liposomes were made in HKN, and either EDTA (disodium ethylenediaminetetraacetate) or $CaCl_2$ was added to give a final concentration of 10^{-3} M. ^{35}S-Labeled virus was adsorbed at 0 °C for 1 h and then incubated at 37 °C for 2 h. The amount of ^{35}S-labeled protein incorporated into liposomes was counted.

red cells and in the pH optimum of hemolysis may reflect basic differences in these processes, differences in the strains used, or differences in the buffers used. When the pH dependence of Sendai virus membrane fusion with liposomes was assayed in Mes–Hepes buffer, the results were similar to those obtained in PBS which are shown in Figure 4. When fusion was assayed in Mes buffer in the pH range 4–7, however, there appeared to be an optimum between pH 5.5 and 7.0. This difference is partly a result of the fact that the amount of fusion obtained at pH 7.0 in Mes buffer was not as great as that obtained at pH 7.0 in Mes–Hepes buffer and in PBS. The abrupt increase in the amount of fusion at pH 4.0 occurred in Mes buffer as well as in Mes–Hepes buffer and in PBS.

It is not clear why the amount of fusion increases at the two extremes of pH. While the charge on the liposomes is probably not neutralized at the pHs studied since it is due to the gangliosides' sialic acid which has a pK_a in the range 2.6–2.75 (Ledeen & Yu, 1976), the charge on the viral surface may be neutralized which would reduce the energy barrier to the very close approach of the bilayers needed for fusion to occur. If this were so, viruses should fuse in the low pH range according to their isoelectric points. The isoelectric point of influenza WSN is 6.0–6.5 (Lakshmi & Schulze, 1978), and influenza WSN causes hemolysis with a maximum around pH 6.0 (Lenard & Miller, 1981). Tischer (1962) measured the pH dependence of hemagglutination of different influenza subtypes and of Sendai virus and suggested the results were related to the isoelectric points of the viruses. She found a maximum for influenza A/PR8 hemagglutination at pH 5.35 and for Sendai virus at pH 4.1. Influenza A/PR8 causes hemolysis and phospholipid transfer with liposomes at pH 5.2 (Maeda et al., 1981b). By analogy, the abrupt increase in Sendai virus fusion should occur just below pH 4.1, as it does. Another probable effect of pH extremes would be to destabilize the membranes. It has been reported that influenza virus is inactivated within 20 s at pH 5.2 and suggested that the acidic residues on the influenza HA_2 protein are neutralized at pH 5.2 (Maeda et al., 1981b). On the other hand, it is possible that pH extremes trigger changes in the conformation of viral proteins which could relate to fusion or that changes in the proton gradient across the membrane could be related to the mechanism of fusion. There are no comparable data on the effect of pH upon fusion of Sendai virus membranes with red cells, because red cells lyse below pH 5 and above pH 10 even in the absence of virus (Neurath, 1965). Further work is needed to determine whether fusion at the pH extremes proceeds by a mechanism at all related to fusion at more physiologic pHs and whether it is comparable to the fusion reported for other virus groups (Helenius et al., 1980; Maeda et al., 1981b; Väänänen et al., 1981) at low pH.

Effect of EDTA and Ca^{2+} upon Sendai Virus Membrane Fusion. Liposomes were made in HKN and either EDTA or $CaCl_2$ was added to determine if Ca^{2+} has any effect upon Sendai virus membrane fusion with liposomes. As shown in Table I, neither EDTA nor Ca^{2+} had a significant effect upon the amount of fusion. This is consistent with the finding that Sendai virus membranes can fuse with chicken erythrocyte membranes in the presence of EGTA (Volsky & Loyter, 1978). Concentrations of Ca^{2+} higher than 0.5 mM have been reported to inhibit fusion of human erythrocytes with Sendai virus membranes (Apostolov & Poste, 1972). The inhibitory effect of Ca^{2+} must be upon the erythrocyte rather than upon the virus, since even when the Ca^{2+} concentration is raised to 10 mM, Ca^{2+} does not inhibit Sendai virus membrane fusion with liposomes. Virus membrane fusion appears to be the exception to the general rule that membrane fusion requires Ca^{2+}. The reason for this is unknown, but one possible explanation might be that the viral receptor–ligand binding can fulfill some of the same functions as Ca^{2+}.

In conclusion when the conditions that optimize Sendai virus membrane fusion with host cells and with red cells are compared to the conditions that optimize fusion with receptor-containing liposomes, the conditions are the same. Many of these conditions should affect proteins rather than the mixture of lipids present in the viral and liposomal membranes. Since there are no proteins present in the liposomal membrane, the proteins that determine the optimal conditions for membrane fusion must be the viral proteins.

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Chemical Modification of Actin. Acceleration of Polymerization and Reduction of Network Formation by Reaction with *N*-Ethylmaleimide, (Iodoacetamido)tetramethylrhodamine, or 7-Chloro-4-nitro-2,1,3-benzoxadiazole†

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ABSTRACT: We examined the properties of rabbit skeletal muscle actin labeled at Cys-373 with *N*-ethylmaleimide or with (iodoacetamido)tetramethylrhodamine, and of *N*-ethylmaleimide–actin further modified with 7-chloro-4-nitro-2,1,3-benzoxadiazole (which primarily labels Lys-372). All three derivatives polymerize more rapidly than unlabeled actin. As measured by fluorescence photobleaching recovery and low-shear viscometry, all three also show a lower extent of network formation relative to native actin. *N*-Ethylmaleimide has a much smaller effect on the rate of polymerization and on network formation than do the other two derivatives. We suggest that chemical modification of actin with these compounds may stabilize nuclei, accounting for the acceleration of polymerization. Stabilization of nuclei also reduces the average filament length at equilibrium, thereby reducing the extent of network formation. We note a parallel between these results and the effects that cytochalasin and capping proteins have on the polymerization of actin.

The polymerization of actin involves several processes, including formation of oligomers (nuclei) from monomeric actin, elongation of the nuclei into long filaments, and end to end reactions between filaments (Oosawa & Asakura, 1975; Pollard & Craig, 1982). In addition, filaments can interact to form networks in solution (Kasai et al., 1960; Maruyama et al., 1974; Tait & Frieden, 1982b). These processes may be important in the regulation of cell motility and the structure of the cytoskeleton and can all be affected by many recently discovered proteins (Craig & Pollard, 1982; Korn, 1982; Schliwa, 1981).

Chemical modification of actin is one approach to an understanding of how these events occur. Some modifications slow or prevent polymerization. For example, Bender et al. (1976) showed that modification of actin at Tyr-53 with 5-diazonium-1*H*-tetrazole completely prevented polymerization. Actin nitrated at Tyr-69 polymerized more slowly than native actin, and attachment of a dansyl group at this residue completely prevented polymerization (Chantler & Gratzer, 1975). Carbethoxylation of several histidine residues also prevented polymerization (Hegyi et al., 1974). Until now, there have been no reports of chemically modified actins which polymerize more rapidly than native actin, nor of derivatives which have altered properties in reactions such as annealing or network formation.

There have been many studies of derivatives of actin labeled at Cys-373. All studies so far have concluded that modification of this residue does not alter the rate or extent of polymerization or other properties of the actin (e.g., Lusty & Fasold, 1969; Stone et al., 1970; Lin, 1978; Ikkai et al., 1979; Thomas et al., 1979; Wang & Taylor, 1980; Taylor et al., 1981). However, there are a few discrepancies in these results. Although Stone et al. (1970) found that modification of actin with a maleimide-based spin-label did not impair its ability to polymerize, they also said that "occasionally spin-labeled samples showed an enhanced rate of viscosity development". Wang & Taylor (1981) found that actin labeled with *N*-ethylmaleimide (MalNEt),[1] eosin–maleimide, or 5-(iodo-

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[1] Abbreviations: FPR, fluorescence photobleaching recovery; IATR, (iodoacetamido)tetramethylrhodamine; MalNEt, *N*-ethylmaleimide; NBD-Cl, 7-chloro-4-nitro-2,1,3-benzoxadiazole; rhodamine–actin, actin labeled at Cys-373 with IATR; Tris, tris(hydroxymethyl)aminomethane.

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