JOURNAL OF VIROLOGY, Feb. 1986, p. 678–683
0022-538X/86/020678-06$02.00/0

Vol. 57, No. 2

Dissociation of Newly Synthesized Sendai Viral Proteins from the Cytoplasmic Surface of Isolated Plasma Membranes of Infected Cells

SUSAN E. CALDWELL AND DOUGLAS S. LYLES*

Department of Microbiology and Immunology, Wake Forest University Medical Center, Winston-Salem, North Carolina 27103

Received 11 January 1985/Accepted 24 October 1985

The interaction of Sendai viral proteins with the membranes of infected cells during budding of progeny virions was studied. BHK cells infected with Sendai virus were labeled with [^{35}S]methionine, and the plasma membranes were purified on polycationic polyacrylamide beads. The isolated membranes were incubated with various agents which perturb protein structure to dissociate viral proteins from the membranes. Incubation of membranes with thiocyanate and guanidine removed both the M and nucleocapsid proteins. Urea (6 M) removed the nucleocapsid proteins but removed M protein only in the presence of 0.1 or 1.0 M KCl. In contrast, high salt concentrations alone eluted only the M protein, leaving the nucleocapsid proteins completely membrane bound. About 65% of the M protein was eluted in the presence of 4 M KCl. The remaining membrane-associated M protein was resistant to further extraction by 4 M KCl. Thus, M protein forms two types of interaction with the membrane, one of them being a more extensive association with the membrane than the other.

The maturation of enveloped viruses such as paramyxoviruses involves the association of newly synthesized viral proteins with the plasma membrane of the infected cell before budding. We investigated the associations of the newly synthesized proteins of Sendai virus with the plasma membrane during this final stage of virion morphogenesis. Sendai virus has two glycoproteins present on the surface of the infected cell and the virion envelope. One glycoprotein, designated HN, possesses both hemagglutinating and neuraminidase activities and mediates the initial attachment of virions to sialic acid-containing receptors on the cell surface (25, 30). The other glycoprotein, designated F, allows the viral envelope to fuse with the cell plasma membrane (12, 25) and is present in the form of an inactive precursor, F_0, in most infected tissue culture cells. Both HN and F are transmembrane proteins (2, 19) bound to the membrane by hydrophobic sequences that span the lipid bilayer (25, 26). The mechanism by which the internal viral components interact with the host cell plasma membrane is less well understood. The nucleocapsid consists of single-stranded RNA associated with a major protein subunit, NP, and two other proteins, P and L, thought to be involved in RNA polymerase activity. This structure probably binds to plasma membranes by interacting with an internal viral membrane or matrix protein, designated M. The evidence for this association is that (i) M is bound to nucleocapsids after detergent disruption of purified virions but can be removed by treatment with high salt concentrations (22, 27, 33); (ii) M can be chemically cross-linked to NP in intact virions (21); (iii) nucleocapsids of a temperature-sensitive mutant of Sendai virus defective in HN and M do not bind to host cell plasma membranes at the nonpermissive temperature (32); and (iv) electron microscopy of the cytoplasmic surface of the plasma membrane of Sendai virus-infected cells has revealed nucleocapsids associated with structures that appear to be composed of M protein (5).

* Corresponding author.

We previously studied the interactions of Sendai viral proteins with the cytoplasmic surface of erythrocyte membranes with which the virus had fused (6) as a model for membrane interactions after virus penetration into host cells. Inside-out vesicles generated from the erythrocyte membranes containing viral proteins were treated with various agents that perturb proteins to remove the internal viral proteins from the cytoplasmic (outer) surface of the vesicles. With this approach, we found that the M and nucleocapsid proteins of Sendai virus behave as peripheral membrane proteins, whereas the glycoproteins behave as integral membrane proteins (for other examples of this approach, see references 9, 18, and 28). However, the association of viral proteins with membranes was unexpectedly strong, since agents such as high concentrations of salt which disrupt M-nucleocapsid interactions after detergent extraction failed to remove the nucleocapsid from the membrane. Additionally, some reagents removed both M and nucleocapsid proteins, whereas others removed only the nucleocapsid proteins, leaving the M protein associated with the bilayer. None of the agents tested removed the M protein and left the nucleocapsid proteins membrane associated.

The goal of this study was to determine the nature and strength of the forces that bind the internal proteins of Sendai virus to the host cell plasma membrane during budding by attempting to remove the proteins from the membrane. Plasma membranes isolated from Sendai virus-infected BHK cells were treated with agents that perturb proteins and remove internal viral proteins from the cytoplasmic surface of the cell membrane. We concentrated on agents such as guanidine hydrochloride, urea, and potassium thiocyanate, which denature proteins by similar mechanisms and are effective at removing viral proteins from the membrane. The mechanism of action of these agents, referred to as chaotropic activity, involves favoring the exposure of buried hydrophobic residues of proteins by preventing an ordered water structure from forming around these residues (31). We also examined the effects of KCl, which has no

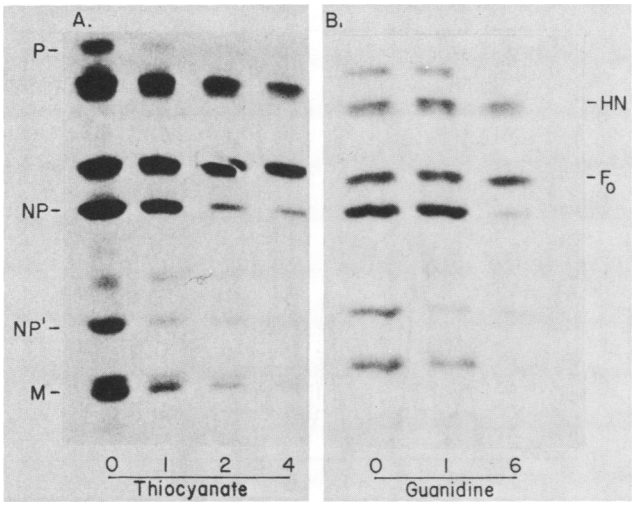

FIG. 1. Treatment of BHK cell plasma membranes containing Sendai viral proteins with thiocyanate (A) or guanidine (B). BHK cells infected with Sendai virus were pulsed (10 min) with [^{35}S]methionine and then chased (1 h) with unlabeled medium. The plasma membranes were purified and treated with 0, 1, 2, or 4 M thiocyanate (A) or 0, 1, or 6 M guanidine (B) in 10 mM Tris (pH 7.4). The membranes were then solubilized in sample buffer and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography.

chaotropic activity but can dissociate ionic bonds. The results indicate that the extent of association of newly synthesized nucleocapsids with plasma membranes is similar to that previously observed after viral envelope fusion (6). In particular, the nucleocapsid appears to be firmly bound to the cytoplasmic surface of the membrane. In contrast, high salt concentrations remove most of the M protein from the membrane before budding without simultaneous removal of the nucleocapsid proteins.

Confluent monolayers of BHK cells were infected with Sendai virus at a multiplicity of approximately 5 PFU per cell. At 18 h postinfection, the maintenance medium was removed and the cells were labeled for 10 min with methionine-deficient Dulbecco modified Eagle medium containing [^{35}S]methionine (25 μCi/ml, >500 mCi/mmol) and 2.5% fetal bovine serum. After the pulse, the cells were incubated at 37°C for 1 h in Dulbecco modified Eagle medium containing unlabeled methionine (0.2 mM) and 3% fetal bovine serum to allow newly synthesized viral proteins to accumulate in plasma membranes to maximum levels (3). At the end of the chase, the cells were placed on ice. The cells were harvested by incubating them on ice for 10 min in 0.9% sodium chloride containing 1 mM EDTA. Plasma membranes were purified from 3×10^6 to 6×10^6 cells essentially as described by Bowen and Lyles (2) through a modification of the technique described by Cohen et al. (7). Briefly, a 50% suspension of cells was mixed with a 20% suspension of polycationic polyacrylamide beads (Affi-Gel 731; Bio-Rad Laboratories) in sucrose-acetate buffer. The cells bound noncovalently to the beads, and four to five successive washes in the same buffer removed unattached cells. The beads were vigorously vortexed in 100 to 200 μl of buffer for 30 s to lyse the attached cells, followed by the immediate addition of 10 mM Tris buffer (pH 7.4) containing aprotinin (10 μg/ml; Sigma Chemical Co.). The plasma membranes remained bound by their outer surfaces to the beads. The beads were allowed to settle at $1 \times g$ or, alternatively, were sedimented for 15 s at $170 \times g$ in a refrigerated model PR-J International Centrifuge and were washed four times with 10 mM Tris to remove cellular debris.

The washed beads with plasma membranes on their surfaces were divided into 50-μl samples for treatment with various agents which perturb protein structure. Each sample of beads was mixed with 0.5 ml of a given agent in 10 mM Tris (pH 7.4) containing 10 μg of aprotinin per ml and incubated on ice for 30 min. The beads were pelleted briefly, the supernatant was removed, and the beads were washed once in 1 ml of 10 mM Tris. The supernatant from the 30-min incubation and the subsequent wash were mixed and centrifuged at 50,000 rpm for 1 h in an SW50.1 rotor at 5°C to pellet any plasma membranes removed from the beads during treatment with an eluting agent. After centrifugation, supernatants were precipitated with 10% (wt/wt) trichloroacetic acid (Fisher Scientific Co.) after 100 μg of bovine serum albumin (Sigma) had been added as a carrier. Samples were centrifuged at $600 \times g$ for 30 min, washed twice with butanol (Fisher), and evaporated to dryness under a stream of nitrogen. Membranes remaining on the beads were solubilized in electrophoresis sample buffer (15), boiled for 2 min in a water bath, and then centrifuged for 20 s in an Eppendorf microcentrifuge to pellet the beads. Membranes were analyzed by electrophoresis on 7.5% polyacrylamide gels containing sodium dodecyl sulfate as described by Laemmli (15). The gels were processed for fluorography and exposed to preexposed Kodak SB-5 X-ray film (17). Fluorograms were scanned with a soft-laser densitometer (Biomed Instruments, Inc.) with digital integration.

Figure 1 shows the labeled viral proteins associated with isolated plasma membranes after treatment with potassium thiocyanate (Fig. 1A) or guanidine hydrochloride (Fig. 1B). The internal viral proteins, P, NP, and M, were all removed from the membranes by 2 or 4 M potassium thiocyanate and by 6 M guanidine hydrochloride, confirming their behavior as peripheral membrane proteins. NP', a proteolytic fragment of NP that is still capable for forming a nucleocapsid structure (11), was also removed from the membranes. In membranes from cells that were labeled and then chased, M frequently appeared as a doublet (Fig. 1A). The upper band is a phosphorylated form of M (16). No differences between phosphorylated and nonphosphorylated forms of M were noted in this or subsequent experiments with regard to their removal from plasma membranes. The behavior of the L protein (not shown in Fig. 1) was generally similar to that of the P protein. In contrast to the internal viral proteins, the viral glycoproteins, HN and F_0, remained associated with the membranes after treatment with potassium thiocyanate or guanidine hydrochloride, as expected for integral transmembrane proteins. HN also appeared as two bands which differed in molecular weight owing to posttranslational processing. The lower-molecular-weight form is the one found in virions. Treatment of membranes with 1 M thiocyanate resulted in the partial removal of the internal viral proteins, none of which appeared to be more susceptible to removal than the others. In contrast to treatment with 1 M thiocyanate, M and NP' appeared to be removed to a greater extent by 1 M guanidine than P or NP.

The results shown in Fig. 1 demonstrate that the M protein and nucleocapsid proteins can be removed from the plasma membrane without disrupting the lipid bilayer by using denaturing conditions. These results confirm and extend the results of our previous experiments with erythrocyte membranes with which Sendai virus had fused (6). We also attempted to remove the internal viral proteins from mem-

branes by using nondenaturing conditions, which are frequently used to dissociate peripheral proteins from membranes. Of particular interest were the effects of high salt concentrations. M protein can be dissociated from nucleocapsids by 1 M KCl in the presence of detergents (24); however, no release of viral proteins from erythrocyte membranes with which Sendai virus had fused was detected after treatment with 1 M KCl (6). An unanticipated result was obtained when plasma membranes from virus-infected BHK cells were treated with 1 M KCl. A large fraction of the M protein was removed by high salt concentrations, and this removal was accomplished without any removal of the nucleocapsid (Fig. 2); a densitometer scan of a fluorogram of the gel is shown. Since HN and F_0 are not removed from membranes by treatments that do not disrupt the lipid bilayer, the densitometer gain was adjusted to give equivalent peak heights for these proteins in the two scans to normalize the data for the recovery of membranes. Approximately half of the M protein was removed from the membranes by treatment with 1 M KCl, whereas the amount of NP protein associated with the membranes was unaffected. Most of the P and NP′ proteins were also removed by 1 M KCl treatment.

The concentration dependence of the ability of KCl to remove NP and M from plasma membranes of BHK cells is shown in Fig. 3A and B. The data were quantitated by dividing the amount of NP or M associated with the membranes by the amount of HN plus F_0 to normalize the data for membrane recovery. The ratio for control membranes incubated in 10 mM Tris (pH 7.4) was considered to represent 100% of NP or M associated with the membranes. The amount of NP associated with the membranes was independent of ionic strength even up to 4 M KCl (Fig. 3A). It is likely that the nucleocapsids bound to the plasma membranes remained intact in the presence of 4 M KCl, since little disruption of isolated virion nucleocapsids was observed after treatment with 4 M KCl (unpublished results). A separate experiment was performed to determine whether intact nucleocapsids were released from the membranes by high salt concentrations and had pelleted with the membranes during centrifugation. Purified membranes containing labeled Sendai virus proteins were treated with 4 M KCl; the beads were pelleted, and the supernatants were centrifuged through a discontinuous sucrose gradient to separate any free intact nucleocapsids from membranes removed from the beads during KCl treatment. Radioactive viral proteins sedimenting with intact nucleocapsids were not observed, indicating that the nucleocapsids were still bound to the membranes (data not shown).

The effective range of KCl concentration required to remove the M protein from the membranes was between 1 and 4 M (Fig. 3B). Approximately 65% of the M protein could be removed by 4 M KCl. However, removal of the remaining 35% could not be accomplished either by extending the incubation time in 4 M KCl to 90 min or by repeatedly extracting with 4 M KCl (data not shown). This result suggests that a fraction of the M protein associated with the membranes can be removed by KCl but that the rest is bound to the membranes in such a way as to resist dissociation at high salt concentrations. The M protein that cannot be removed from the plasma membranes of virus-producing cells may be more immediately involved in virus assembly than the more easily extractable M protein. For example, the M protein that remains associated with membranes at high salt concentrations may be directly involved in binding to nucleocapsids.

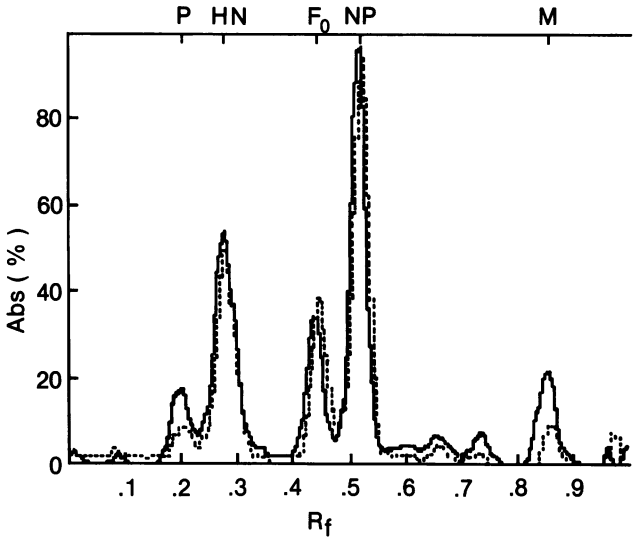

FIG. 2. Densitometric tracing of a fluorogram of BHK cell plasma membranes containing Sendai viral proteins after 1.0 M KCl treatment. Sendai virus-infected BHK cells were pulsed for 10 min with [^{35}S]methionine and then chased for 60 min with unlabeled medium. The plasma membranes were purified and incubated with 10 mM Tris (pH 7.4) or 1.0 M KCl in 10 mM Tris for 30 min at room temperature. The membranes were solubilized in sample buffer and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. The top of the fluorogram is at the left. ——, Control membranes treated with 10 mM Tris (pH 7.4); -----, membranes treated with 1.0 M KCl. Abs (%), % Absorption.

In our previous study of membranes with which Sendai virus had fused, we found that urea selectively removed NP but left M associated with the membranes, in contrast to other chaotropic agents, such as guanidine hydrochloride and potassium thiocyanate (6). It is known that urea is not as effective as guanidine in denaturing proteins (29). Thus, a potential explanation for this result is that the M protein interacts more extensively with membranes than do nucleocapsids, so that harsher conditions are required for the removal of the M protein. It is also possible that the difference between the effects of guanidine and urea may be due to the fact that urea is not ionic, whereas guanidine and thiocyanate are. If the latter possibility were true, then treatment with urea at an elevated ionic strength should mimic the effects of guanidine or thiocyanate. Such a result is shown in Fig. 3C and D, in which BHK cell plasma membranes were treated with 6 M urea in the presence of various concentrations of KCl. Treatment of plasma membranes from virus-infected cells with urea alone removed primarily NP (about 65% of the total), whereas relatively little M protein was removed (about 25% of the total). However, treatment with urea in the presence of 0.1 or 1.0 M KCl resulted in the removal of about 60% of the M protein. These data suggest that denaturation of nucleocapsids with urea is sufficient to remove NP from membranes but that M remains associated with membranes through a salt-labile, presumably ionic, interaction. We occasionally noted that 50 to 60% of NP remained associated with the membranes after treatment with urea in the presence of 1 M KCl. Incubation in high salt concentrations causes isolated nucleocapsids to assume a tightly coiled configuration (11), which may partially stabilize nucleocapsids against denaturation by urea. Likewise, the M protein could not be completely removed by the combination of urea and KCl (Fig. 3D), although in

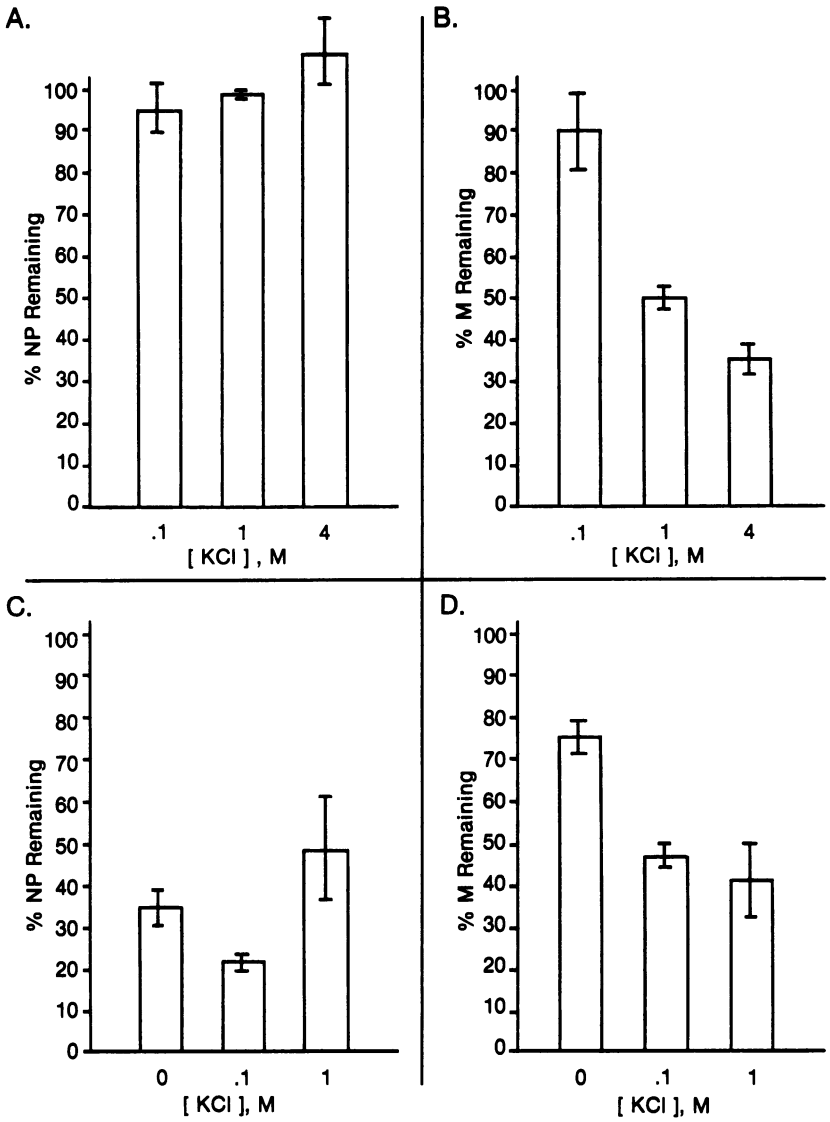

FIG. 3. Sendai viral M and NP proteins remaining membrane associated after treatment with 6 M urea or KCl or both. Sendai virus-infected BHK cells were pulsed for 10 min with [^{35}S]methionine and chased for 1 h with unlabeled medium. The plasma membranes were purified and treated with the indicated concentrations of urea or KCl or both. Samples treated with 4 M KCl were incubated at room temperature. The membranes were solubilized and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. The radiolabeled viral proteins were quantitated by densitometry of the fluorograms. The amount of NP or M protein associated with the membranes was divided by the amount of HN plus F_0 to normalize the data for membrane recovery. The ratio for control membranes represented 100% of the NP or M protein associated with the membranes. The data are expressed as the mean ± standard error of the mean for three to six experiments. (A) NP protein remaining membrane bound after treatment with the indicated concentrations of KCl. (B) M protein remaining membrane bound after treatment with the indicated concentrations of KCl. (C) NP protein remaining membrane bound after treatment with 6 M urea in the presence of the indicated concentrations of KCl. (D) M protein remaining membrane bound after treatment with 6 M urea in the presence of the indicated concentrations of KCl.

the presence of urea 50 to 60% of the M protein could be removed by much lower salt concentrations (0.1 to 1 M KCl) than in the absence of urea. Therefore, the interaction of the M protein with the plasma membrane appears to be maintained by a combination of ionic interactions such that the native protein conformation stabilizes the association with the membrane, whereas the denatured M protein remains bound to the membrane by more easily dissociated ionic bonds.

It is unlikely that the fraction of the M protein that was more readily dissociated from the membranes resulted from the artifactual association of the M protein with the membranes, e.g., during cell fractionation. We tested for the artifactual association of viral proteins with plasma membranes during fractionation by an experiment analogous to that performed by Atkinson et al. (1) with plasma membranes from cells infected with vesicular stomatitis virus. Polycationic beads containing plasma membranes isolated from unlabeled cells (at 75% the normal ratio of cells to beads) were mixed with radiolabeled cells (the remaining 25% of the total cells), which are able to attach to unoccupied areas of the beads. After lysis of the labeled cells and reisolation of the plasma membranes, the label distribution among the viral proteins was the same as that observed when

the labeled and unlabeled cells were mixed before membrane isolation. If there were an artifactual association of one or more of the viral proteins with the plasma membranes, then this protein would be preferentially labeled by this procedure, since the labeled protein would have a large area of unlabeled membrane with which to associate. As an additional control for the artifactual association of viral nucleocapsids with plasma membranes, we determined that nucleocapsids isolated from virions do not associate with isolated plasma membranes regardless of whether they have M protein bound to them or not (2).

The results presented here confirm our previous observation that Sendai viral proteins, including both the M protein and the nucleocapsid, interact extensively with the plasma membrane of the host cell. The evidence for this is that the viral proteins must be denatured to be released from the membrane. Conditions which can remove many peripheral proteins from membranes, such as extremes of ionic strength or low concentrations of denaturants, do not release viral nucleocapsids from the membrane. This unexpectedly firm attachment of nucleocapsids to host cell membranes, first observed after viral envelope fusion (6), appears to originate in the process of viral maturation by budding, as shown by the results presented here. Based upon the kinetics of incorporation of NP and P into plasma membranes and virions (3, 13), Bowen and Lyles (3) postulated that the nucleocapsids associated with isolated plasma membranes represent nascent nucleocapsids in the process of being assembled. If this is so, then the association of the newly forming nucleocapsids with the plasma membranes is not notably weaker than that of the mature virion nucleocapsids examined in our previous study (6). Thus, nucleocapsids appear to be firmly attached to membranes at the early stages of virion assembly as well as at the final stages of virion uncoating by fusion with host cell membranes. It is reasonable to assume that the attachment of nucleocapsids to membranes is also preserved in the virion envelope. Although we have not tested this point, many electron micrographs show a proximity of nucleocapsids to envelopes of Sendai virions.

It was expected that the M protein would be found to interact more extensively than the nucleocapsid with the host cell plasma membrane. The M proteins of other viruses, such as influenza virus and vesicular stomatitis virus have been shown to interact directly with phospholipid bilayers in the absence of other proteins (4, 10, 34) and, in the case of vesicular stomatitis virus, the M protein can be chemically cross-linked to lipids, hydrophobic membrane probes, or viral glycoproteins in intact virions (8, 20, 23). Our previous data indicating that urea can remove the Sendai virus nucleocapsid from membranes without removing the M protein (6) also led us to expect that the M protein would be more extensively membrane associated than the nucleocapsid. However, data presented in this paper show that it is not possible at present to generalize whether the M protein or the nucleocapsid interacts more extensively with host cell membranes. This interpretation is based on evidence that urea can remove most of the nucleocapsid without removing the M protein, whereas KCl can dissociate most of the M protein without removing the nucleocapsid. The mechanism by which the nucleocapsid remains attached to the membrane after the removal of most of the M protein by KCl is of particular interest. It is possible that the M protein is not responsible for nucleocapsid binding to the membrane. However, it is more likely that the fraction of the M protein resistant to KCl extraction is involved in nucleocapsid binding to the membrane. If the M protein that is resistant to KCl extraction is responsible for nucleocapsid binding, this would also suggest that the interaction with the nucleocapsid is necessary for the stable attachment of the M protein to the cytoplasmic surface of the host cell membrane. This hypothesis is supported by studies showing that the M protein does not associate with membranes in the case of temperature-sensitive mutants of vesicular stomatitis virus that fail to assemble nucleocapsids at the nonpermissive temperature (14). Thus, the association of internal viral proteins with the cytoplasmic surface of the host cell plasma membrane may be a cooperative process requiring the simultaneous presence of both the M protein and the nucleocapsid.

This work was supported by Public Health Service grant AI15892 from the National Institutes of Health.

We extend our appreciation to Joyce Hill for preparing this manuscript.

LITERATURE CITED

1. **Atkinson, P. H., S. A. Moyer, and D. F. Summers.** 1976. Assembly of vesicular stomatitis virus glycoprotein and matrix protein into HeLa cell plasma membranes. J. Mol. Biol. **102**:613–631.
2. **Bowen, H. A., and D. S. Lyles.** 1981. Structure of Sendai viral proteins in plasma membranes of virus-infected cells. J. Virol. **37**:1079–1082.
3. **Bowen, H. A., and D. S. Lyles.** 1982. Kinetics of incorporation of Sendai virus proteins into host plasma membranes and virions. Virology **121**:1–11.
4. **Bucher, D. J., I. G. Kharitonenkov, J. A. Zakomirdin, V. B. Grigoriev, S. M. Klimenko, and J. F. Davis.** 1980. Incorporation of influenza virus M-protein into liposomes. J. Virol. **36**:586–590.
5. **Büechi, M., and T. Bächi.** 1982. Microscopy of internal structures of Sendai virus associated with the cytoplasmic surface of host membranes. Virology **120**:349–359.
6. **Caldwell, S. E., and D. S. Lyles.** 1981. Interaction of Sendai virus proteins with the cytoplasmic surface of erythrocyte membranes following viral envelope fusion. J. Biol. Chem. **256**:4838–4842.
7. **Cohen, C. M., D. I. Kalish, B. S. Jacobson, and D. Branton.** 1977. Membrane isolation on polylysine-coated beads. J. Cell Biol. **75**:119–134.
8. **Dubovi, E. J., and R. R. Wagner.** 1977. Spatial relationships of the proteins of vesicular stomatitis virus: induction of reversible oligomers by cleavable protein cross-linkers and oxidation. J. Virol. **22**:500–509.
9. **Gerace, L., Y. Ottaviano, and C. Kondor-Koch.** 1982. Identification of a major polypeptide of the nuclear pore complex. J. Cell Biol. **95**:826–837.
10. **Gregoriades, A., and B. Frangione.** 1981. Insertion of influenza M protein into the viral lipid bilayer and localization of site of insertion. J. Virol. **40**:323–328.
11. **Heggeness, M. H., A. Scheid, and P. W. Choppin.** 1980. Conformation of the helical nucleocapsids of paramyxoviruses and vesicular stomatitis virus: reversible coiling and uncoiling induced by changes in salt concentration. Proc. Natl. Acad. Sci. USA **77**:2631–2635.
12. **Homma, M., and M. Ohuchi.** 1973. Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural difference of Sendai viruses grown in eggs and tissue culture cells. J. Virol. **12**:1457–1465.
13. **Kingsbury, D. W., C. M. Hsu, and K. G. Murti.** 1978. Intracellular metabolism of Sendai virus nucleocapsids. Virology **91**:86–94.
14. **Knipe, D. M., D. Baltimore, and H. F. Lodish.** 1977. Maturation of viral proteins in cells infected with temperature-sensitive mutants of vesicular stomatitis virus. J. Virol. **21**:1149–1158.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the

assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **Lamb, R. A., and P. W. Choppin.** 1977. The synthesis of Sendai virus polypeptides in infected cells. III. Phosphorylation of polypeptides. Virology **81**:382–397.
17. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56**:335–341.
18. **Lively, M. O., and K. A. Walsh.** 1983. Hen oviduct signal peptidase is an integral membrane protein. J. Biol. Chem. **258**:9488–9495.
19. **Lyles, D. S.** 1979. Glycoproteins of Sendai virus are transmembrane proteins. Proc. Natl. Acad. Sci. USA **76**:5621–5625.
20. **Mancarella, D. A., and J. Lenard.** 1981. Interactions of wild-type and mutant M protein of vesicular stomatitis virus with viral nucleocapsid and envelope in intact virions. Evidence from [^{125}I]iodonaphthyl azide labeling and specific crosslinking. Biochemistry **20**:6872–6877.
21. **Markwell, M. A. K., and C. F. Fox.** 1980. Protein-protein interactions within paramyxoviruses identified by native disulfide bonding or reversible chemical cross-linking. J. Virol. **33**:152–166.
22. **McSharry, J. J., R. W. Compans, H. Lackland, and P. W. Choppin.** 1975. Isolation and characterization of the nonglycosylated membrane protein and a nucleocapsid complex from the paramyxovirus SV5. Virology **67**:365–374.
23. **Pepinsky, R. B., and V. M. Vogt.** 1979. Identification of retrovirus matrix proteins by lipid-protein cross-linking. J. Mol. Biol. **131**:819–837.
24. **Scheid, A., and P. W. Choppin.** 1973. Isolation and purification of the envelope proteins of Newcastle disease virus. J. Virol. **11**:263–271.
25. **Scheid, A., and P. W. Choppin.** 1974. Identification of the biological activities of paramyxovirus glycoproteins: activation of cell fusion, hemolysis and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475–490.
26. **Scheid, A., and P. W. Choppin.** 1977. Two disulfide-linked polypeptide chains constitute the active F protein of paramyxoviruses. Virology **80**:54–66.
27. **Shimizu, K., and N. Ishida.** 1975. The smallest protein of Sendai virus: its candidate function of binding nucleocapsid to envelope. Virology **67**:427–437.
28. **Steck, T. L., and J. Yu.** 1973. Selective solubilization of proteins from red blood cell membranes by protein perturbants. J. Supramol. Struct. **1**:220–232.
29. **Tanford, C.** 1968. Protein denaturation. Adv. Protein Chem. **23**:121–282.
30. **Tozawa, H., M. Watanabe, and N. Ishida.** 1973. Structural components of Sendai virus. Serological and physicochemical characterization of hemagglutinin subunits associated with neuraminidase activity. Virology **55**:242–253.
31. **Von Hippel, P. H., and T. Schleich.** 1969. Ion effects on the solution structure of biological macromolecules. Acc. Chem. Res. **2**:257–265.
32. **Yoshida, T., Y. Nagai, K. Maeno, M. Iinuma, M. Hamaguchi, T. Matsumoto, S. Nagayoshi, and M. Hoshino.** 1979. Studies on the role of M protein in virus assembly using a ts mutant of HVJ (Sendai virus). Virology **92**:139–154.
33. **Yoshida, T., Y. Nagai, S. Yoshii, K. Maeno, and T. Matsumoto.** 1976. Membrane (M) protein of HVJ (Sendai virus): its role in virus assembly. Virology **71**:143–161.
34. **Zakowski, J. J., W. A. Petri, Jr., and R. R. Wagner.** 1981. Role of matrix protein in assembling the membrane of vesicular stomatitis virus: reconstitution of matrix protein with negatively charged phospholipid vesicles. Biochemistry **20**:3902–3907.