Structure of Sendai Viral Proteins in Plasma Membranes of Virus-Infected Cells

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Purified plasma membranes attached to polycationic polyacrylamide beads by their external surface were isolated from BHK cells infected with Sendai virus. Each of the viral proteins could be identified in the membranes of infected cells. Proteolysis with trypsin, which digests only the cytoplasmic surface of these membranes (because the external surface is protected by its attachment to beads), revealed that the internal proteins, L, P, NP, and M, were present on the cytoplasmic surface of the membrane and that small segments of the viral envelope glycoproteins, HN and F_0, were partially exposed on the cytoplasmic surface. Since the major portions of HN and F_0 are known to be present on the external membrane surface, these glycoproteins are transmembrane proteins before Sendai virus budding in infected cells.

Before maturation of paramyxoviruses by budding, the two viral envelope glycoproteins, HN and F_0, are inserted into the host cell plasma membrane (3). The F glycoprotein is composed of two disulfide-linked subunits, F_1 and F_2, generated by proteolytic cleavage of an inactive precursor, F_0 (16). The internal components of the virus include a nonglycosylated envelope protein, M, and a nucleocapsid composed of a single major subunit, NP, and two proteins, P and L, associated with virion transcriptase activity (nomenclature of proteins is discussed in references 14 and 16). These internal virion components must interact with the viral envelope proteins in the budding process so that viral proteins are selectively incorporated into the envelope.

The envelope glycoproteins of Sendai virus are also incorporated into cell membranes by the process of fusion of the virus envelope with the plasma membrane (1), a process normally associated with virus penetration into host cells (6, 15). It has recently been demonstrated that in erythrocyte membranes with which Sendai virus has fused, a small segment of the HN and F_1 glycoproteins spans the membrane lipid bilayer and is exposed on the inside surface (11). We have prepared plasma membranes from virus-infected cells by the technique of Cohen et al. (4) which selectively exposes the cytoplasmic surface of the membrane. The preparation involves the attachment of virus-infected cells to polycationic polyacrylamide beads with subsequent lysis of the cells. The plasma membranes are retained with the external surface of the membrane attached to the bead and the cyto-

plasmic surface exposed (4). The data presented here show that the two Sendai virus glycoproteins have a small segment exposed on the cytoplasmic surface of the host plasma membrane and therefore span the membrane before budding in virus-infected cells. In addition, the presence of internal viral proteins on the cytoplasmic surface of the host cell plasma membrane is demonstrated.

Stocks of Sendai virus (Z strain) were grown in embryonated chicken eggs. Labeled virus was grown in Madin-Darby bovine kidney (MDBK) cells in the presence of [^{35}S]methionine (10 µCi/ml; New England Nuclear Corp.) and purified as described previously (14). Baby hamster kidney (BHK) cells were maintained in Dulbecco-modified Eagle medium (DMEM) with 10% newborn calf serum. BHK cells were infected with Sendai virus at approximately 5 PFU per cell. At 18 h postinfection, the medium was removed and replaced with medium containing either [^{35}S]methionine (10 µCi/ml; >400 Ci/mmol) or [^{3}H]glucosamine (50 µCi/ml; 10 to 30 Ci/mmol) (both from New England Nuclear Corp.). After incubation for 2 h at 37°C, cells were washed and lifted by incubation in 1 mM EDTA–0.9% NaCl for 30 min at room temperature, and plasma membranes were isolated on Affi-gel 731 beads (Bio-Rad Laboratories) essentially as described by Cohen et al. (4). However, attachment of cells to beads was carried out by suspending the cells in approximately 10 times their volume of attachment buffer and slowly adding an equal volume of a 10% suspension of beads. The final sonication step did not significantly increase the purity of the membranes and was omitted. Pro-

tein determination (10) was carried out after solubilizing protein from beads in 1% sodium dodecyl sulfate–0.15 M NaCl incubated for 2 min at 100°C. Recovery and purity of plasma membranes were assessed by Na^+-K^+ ATPase (19) activity or by surface-specific iodination with ^{125}I (12) and were similar to those described by Cohen et al. for HeLa cells (10 to 30% yield, 5- to 20-fold increase in specific activity of either marker). NADPH-cytochrome c reductase, an enzymatic marker for endoplasmic reticulum (18), was <2% of total cellular activity and was usually undetectable.

To determine whether segments of the viral glycoproteins were exposed on the cytoplasmic surface, inside-out membranes from uninfected and Sendai virus-infected BHK cells labeled with [^3H]glucosamine were treated with trypsin, which digests only proteins on the exposed surface. The proteolysis products were analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide slab gels, and labeled proteins were detected by fluorography (Fig. 1). Both viral glycoproteins, HN and F_0, were partially protected from trypsin digestion. However, digestion of the cytoplasmic surface of the membrane decreased their molecular weights by 1,000 to 2,000. Thus, a small portion of these glycoproteins is exposed on the cytoplasmic surface. Since a major portion of both glycoproteins is known to be exposed on the external surface of the membrane, these results imply that the Sendai virus glycoproteins span the membrane lipid bilayer. This transmembrane orientation was present in all host cells we examined. Figure 2 shows a similar digestion of [^{35}S]methionine-labeled inside-out plasma membranes with trypsin. By comparison with proteins from purified Sendai virions, each viral structural protein was present in the plasma membranes of infected cells, as previously described (8). Note that the F_0 of virus grown in BHK cells migrated faster than the F_0 of virus grown in MDBK cells, as has previously been noted by other investigators (9). There was usually no difference in migration of HN of virus grown in BHK versus MDBK cells, although occasionally a faster-migrating form was observed from either cell type (as in BHK cells in Fig. 1), which presumably is due to a difference in host modification that depends upon culture conditions, etc. Both viral glycoproteins, HN and F_0, were partially protected from trypsin digestion, but their molecular weights decreased by approximately 1,000 to 2,000 as shown in Fig. 1.

The internal virion proteins were largely digested by trypsin treatment (Fig. 2), indicating that they are present on the cytoplasmic surface of the membrane. NP was not detectable after
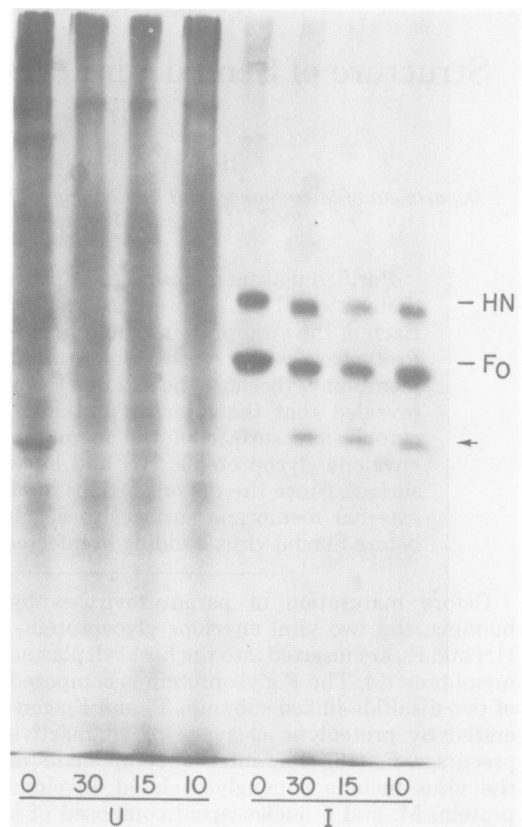

FIG. 1. Proteolysis of plasma membranes of BHK cells labeled with [^3H]glucosamine. Plasma membranes derived from either Sendai virus-infected (I) or uninfected (U) cells (approximately 50 µg of protein) were incubated in 100 µl of 10 mM Tris, pH 7.5, containing trypsin (trypsin-TPCK; Worthington Diagnostics), 50 µg/ml, for the indicated time (minutes) at room temperature with constant gentle agitation. Reactions were stopped by adding 10 µl of soybean trypsin inhibitor (2.5 mg/ml; Sigma Chemical Co.). After removal of the polyacrylamide beads by centrifugation, the entire reaction mixture was electrophoresed immediately on 7.5% polyacrylamide slab gels containing sodium dodecyl sulfate as described previously (7). Gels were processed for fluorography (2) by using Kodak SB-5 film. Positions of the viral glycoproteins HN and F_0 are indicated; the arrow indicates the position of F_1.

trypsin treatment, although proteins with a mobility similar to P and M were still detectable. These were present after trypsin treatment of membranes of uninfected cells and thus are probably host derived. Nonetheless, >80% of P and M relative to HN or F_0 is removed by trypsin treatment, as determined by densitometry.

To test whether the nucleocapsids associated with the plasma membrane preparations were the result of artifactual attachment to beads or
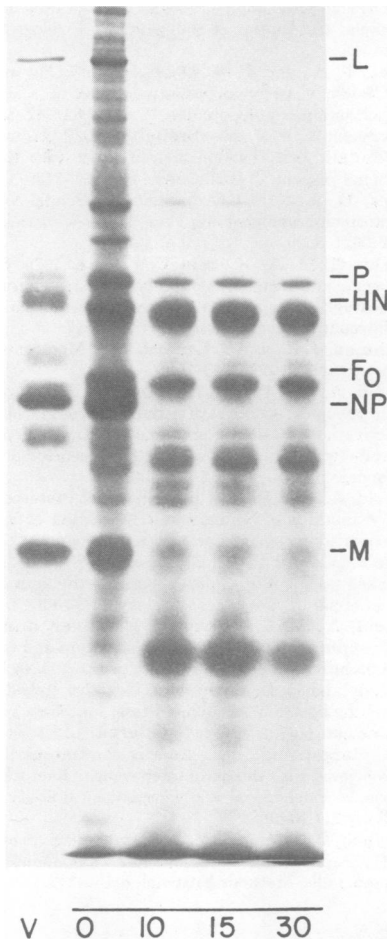
FIG. 2. *Proteolysis of plasma membranes of BHK cells labeled with [^{35}S]methionine. Membranes of Sendai virus-infected cells were incubated with trypsin (final concentration of 50 μg/ml) at room temperature for the indicated time (minutes) as in Fig. 1. Digestion mixtures were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fluorographs of the dried gels are shown. V, Purified virus labeled with [^{35}S]methionine.*

membranes during the isolation procedure, labeled nucleocapsids were purified from infected cells and added to an inside-out plasma membrane preparation from unlabeled cells. A maximum of 3% of the label attached nonspecifically to the beads. This suggests that the nucleocapsid proteins (NP, P, and L) seen in inside-out membrane preparations before digestion with trypsin represented viral protein that was associated with the membrane before its isolation.

The plasma membrane preparation used in this study provides selective exposure of the cytoplasmic surface of the plasma membrane of

virus-infected cells. This inside-out plasma membrane preparation on polyacrylamide beads was originally described for HeLa cells by Cohen et al. (4). The extent of exposure of the original external membrane surface was not quantitated directly by Cohen et al. (4, 5), but it can be estimated to be on the order of 10% based on the extent of labeling of carbohydrates by galactose oxidase and [^{3}H]borohydride (5). The inside-out orientation of plasma membranes on the beads is further supported here by the observation that after trypsinization there was little cleavage of F_0 to F_1 (arrow in Fig. 1). The extent of cleavage of F_0 to F_1 seen in Fig. 1 would suggest that approximately 75 to 80% of the plasma membranes on the beads were oriented so that the original external surface was protected. Additionally, the trypsinization of plasma membranes from uninfected cells labeled with [^{3}H]glucosamine resulted in little loss of label, demonstrating that the original external surface of the membrane, which was selectively labeled by the glucosamine, was protected from digestion. Similar results have been obtained by demonstrating the resistance of label to proteolysis in membranes from cells surface labeled with ^{125}I (data not shown). Conversely, the demonstration in Fig. 2 that internal viral proteins such as NP and M were digested indicates that almost all of the cytoplasmic surface was exposed. Thus, the preparation did not include a significant proportion of sealed, right-side-out vesicles or of intact virus attached to beads, which would protect NP and M from digestion.

It has been shown previously that HN and F_1 are in a transmembrane configuration after fusion of Sendai virus with erythrocyte membranes (11). Thus, a transmembrane structure appears to be a general feature of these proteins, independent of the state of virus maturation. It is likely that the same regions of these proteins are present on the cytoplasmic surface of both the erythrocyte membrane after envelope fusion and the BHK plasma membrane before budding. Since F_1 is derived from the carboxyl terminus of F_0 (16), the fact that F_1 and F_0 have cytoplasmic regions of similar size suggests that the carboxyl terminus of both proteins is exposed on the cytoplasmic membrane surface. A similar structure is also likely for HN, as predicted from the solubility properties of proteolytic fragments of HN (17). The presence of a small cytoplasmic carboxyl segment appears to be characteristic of a large class of viral and cellular glycoproteins (reviewed in reference 13).

The viral nucleocapsid appears to associate specifically with the host plasma membrane in contrast to intracellular membranes (8). The interaction of internal virion components, the

nucleocapsid and the M protein, with the cytoplasmic surface of the plasma membrane appears to be preserved in the preparations described here. Thus, it may be feasible to use this membrane preparation to characterize the interactions among the viral nucleocapsid and envelope components in the budding process.

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