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Role of the Vesicular Stomatitis Virus Matrix Protein in Maintaining the Viral Nucleocapsid in the Condensed Form Found in Native Virions

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Vesicular stomatitis virus was extracted with 60 mM octylglucoside in the absence of salts and in the presence of 0.5 M NaCl. The resulting extracted virus particles were examined by electron microscopy, and the proteins present were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Extraction in the absence of salts yielded subviral structures which we cell "skeletons" as originally suggested by Cartwright et al. (J. Gen. Virol. 7:19-32, 1970). The skeletons contained the viral N, M, and L proteins, but they lacked the glycoprotein (G) entirely. Morphologically, the skeletons resembled intact vesicular stomatitis virus but they were slightly longer and smaller in diameter. Like native vesicular stomatitis virus, skeletons were found to have lateral striations spaced 5.0 to 6.0 nm apart along the length of the structure. In contrast to extraction in the absence of NaCl, extraction of vesicular stomatitis virus with 60 mM octylglucoside in the presence of 0.5 M NaCl yielded highly extended viral nucleocapsids in which N was the predominant protein; no M or G proteins could be detected. These results support the view that the M protein is involved in maintaining the nucleocapsid in the compact form found in native virions.

Extraction of vesicular stomatitis virus (VSV) with detergent can have quite different effects on the virus structure depending on the identity of the detergent employed. For example, extraction with 0.1% sodium deoxycholate results in solubilization of the viral envelope and release of the nucleocapsid in an extended state (2, 18). In contrast, extraction with 0.2% Nonidet P-40 yields structures called "skeletons" (2, 3) in which the nucleocapsid remains condensed as it is in the intact virus. Similar structures, which are probably infectious (1), result from the extraction of VSV with Tween 80 plus diethylether (3) or with digitonin (19). Extraction of Sendai virus with 2% Triton X-100 also yields compact structures. In these complexes, the viral nucleocapsid is found associated with protein VP5. Extraction with 2% Triton X-100 in the presence of 1 M KCl, however, releases free Sendai nucleocapsids lacking VP5 (15). These findings support the conclusion that VP5 is responsible for maintaining the Sendai virus nucleocapsid in its condensed state.

Here we report the results of experiments in which VSV was extracted with the detergent octylglucoside (8, 13, 17) in the absence of salts and in the presence of 0.5 M NaCl. As in the case of Sendai virus extracted with Triton X-100, the presence of salt was found to have a dramatic effect on the disassembly of VSV.

VSV suspensions to be extracted with detergent were first dialyzed overnight against 0.01 M Tris-hydrochloride buffer (pH 7.4) and adjusted to a concentration of 1 to 2 mg of viral protein per ml. Preparations to be extracted in the absence of salt were brought to a concentration of 60 mM 1-*O*-*n*-octyl-β-D-glucopyranoside (octylglucoside; Sigma Chemical Co., St. Louis, Mo. or Calbiochem, La Jolla, Calif.) by the addition of an equal volume of 120 mM octylglucoside in 0.01 M Tris-hydrochloride buffer. The turbid suspensions were allowed to incubate for 1 h at room temperature before insoluble structures were harvested by ultracentrifugation as described in Fig. 1. Extraction in the presence of salt was performed at 4°C by adjusting dialyzed virus suspensions in 60 mM octylglucoside, 0.5 M NaCl, and 20% glycerol in 0.01 M Tris-hydrochloride buffer to a concentration of 0.5 to 1.0 mg of viral protein per ml. Extraction was continued for 45 min at 4°C, after which insoluble structures were harvested by ultracentrifugation. The initially turbid virus suspensions became completely clear upon addition of detergent plus salt, and they remained clear throughout the 45-min extraction period at 4°C.

Subviral complexes resulting from octylglucoside extraction were collected by ultracentrif-

ugation and analyzed in the electron microscope and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Extraction in the absence of salt yielded compact structures somewhat similar in appearance to the intact virus (Fig. 1A) but lacking the glycoprotein "spikes"; we call these structures "skeletons" as originally suggested by Cartwright et al. (2). Skeletons are longer and slightly smaller in diameter than intact VSV (Table 1; Fig. 1D), but they have cross striations with nearly the same spacing (5.0 to 6.0 nm) observed in the native virus. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that skeletons contain the VSV L, N, and M proteins in roughly the same proportions found in intact VSV. They lack the glycoprotein entirely, however (Fig. 2, lane 2). This is consistent with the results of Petri and Wagner (13) and Miller et al. (8) which show that the glycoprotein is solubilized when VSV is treated with octylglucoside.

VSV skeletons are found to be structurally stable when stored at 4°C. Skeleton preparations maintained for up to 7 days at 4°C in 0.01 M Tris-hydrochloride buffer (pH 7.4) gave images in the electron microscope and sodium dodecyl sulfate-polyacrylamide gel profiles indistinguishable from those shown in Fig. 1A and Fig. 2, lane 2, respectively.

Extraction of VSV with octylglucoside in the presence of 0.5 M NaCl was found to yield viral nucleocapsids (Fig. 1B). These highly extended structures are clearly distinguishable from skeletons which are more compact and structurally organized. Nucleocapsids produced by octylglucoside-salt extraction contained the viral N and L proteins, but they lacked both the G and M proteins (Fig. 2, lane 3).

The presence of 0.5 M NaCl had the same effect on extraction of VSV with 1% Triton X-100 as with 60 mM octylglucoside. Extraction in the absence of salt yielded skeletons identical in appearance to those shown in Fig. 1A, whereas extraction with 1% Triton X-100 plus 0.5 M NaCl yielded nucleocapsids. As in the case of octylglucoside extraction, skeletons produced with 1% Triton X-100 contained L, N, and M proteins, whereas nucleocapsids contained only N (Fig. 2, lanes 4 and 5). Neither skeletons nor nucleocapsids produced by Triton X-100 extraction contained detectable G protein.

The results reported here support the view that the VSV M protein plays a crucial role in maintaining the viral nucleocapsid in the compact and highly organized state found in intact virions. Other viral proteins could not perform this condensing and organizing function. The glycoprotein (G), for instance, could not be involved because it is absent from skeletons in which the nucleocapsid is tightly condensed. The L protein alone could not be responsible for condensing the nucleocapsid into skeletons because it is present in uncondensed nucleocapsids (Fig. 2, lane 3). These structures do not contract to form skeletons under any conditions yet examined (4). The NS protein could not be involved in nucleocapsid condensation for the same reason; it is present in extended nucleocapsids (W. Newcomb, unpublished data). The viral phospholipids are also unlikely to be involved in maintaining the nucleocapsid in the compact form found in skeletons. The studies of Miller et al. (8) have shown that VSV phospholipids are nearly completely extracted under conditions (50 mM octylglucoside at low ionic strength) that yield virus skeletons. The absence of a viable alternative makes a strong case for involvement of the M protein in maintaining VSV nucleocapsids in the compact state found in skeletons and in intact virions.

Our observations on VSV are quite similar to those reported for Sendai virus by Shimizu and Ishida (15). Extraction of Sendai virus with 2% Triton X-100 in the absence of salt yields condensed structures containing protein VP5 (molecular weight, 35,000), whereas extraction in the presence of 1 M KCl yields extended nucleocapsids lacking VP5. These findings support the view that like the VSV M protein, Sendai virus protein VP5 is involved in condensing and packaging the viral nucleocapsid. Although the function of Sendai virus VP5 and VSV M proteins must clearly be related, important differences also exist. Sendai virus skeletons contain thick nucleocapsid chains folded in an apparently irregular way to form roughly spherical particles (see Fig. 10A of reference 15). In contrast, the cylindrical VSV skeletons are most likely to be formed from a very regular helical arrangement of the nucleocapsid, as suggested by Nakai and Howatson (11) for the intact virus. The interaction of Sendai virus nucleocapsids with VP5, therefore, results in quite different structures from those containing VSV nucleocapsids and M protein.

Perhaps the most interesting feature of the studies described here is the production of VSV skeletons. These structures can be quickly isolated in high yield by gentle extraction of VSV with octylglucoside or with Triton X-100 as described above. Similar structures result from extraction with 0.2% Nonidet P-40 (2), digitonin (19), or Tween 80 plus diethylether (3). The close morphological resemblance of skeletons and intact VSV encourages one to believe that extraction of VSV to produce skeletons does not involve gross rearrangement of the virus structure. Skeletons may, therefore, prove to be most

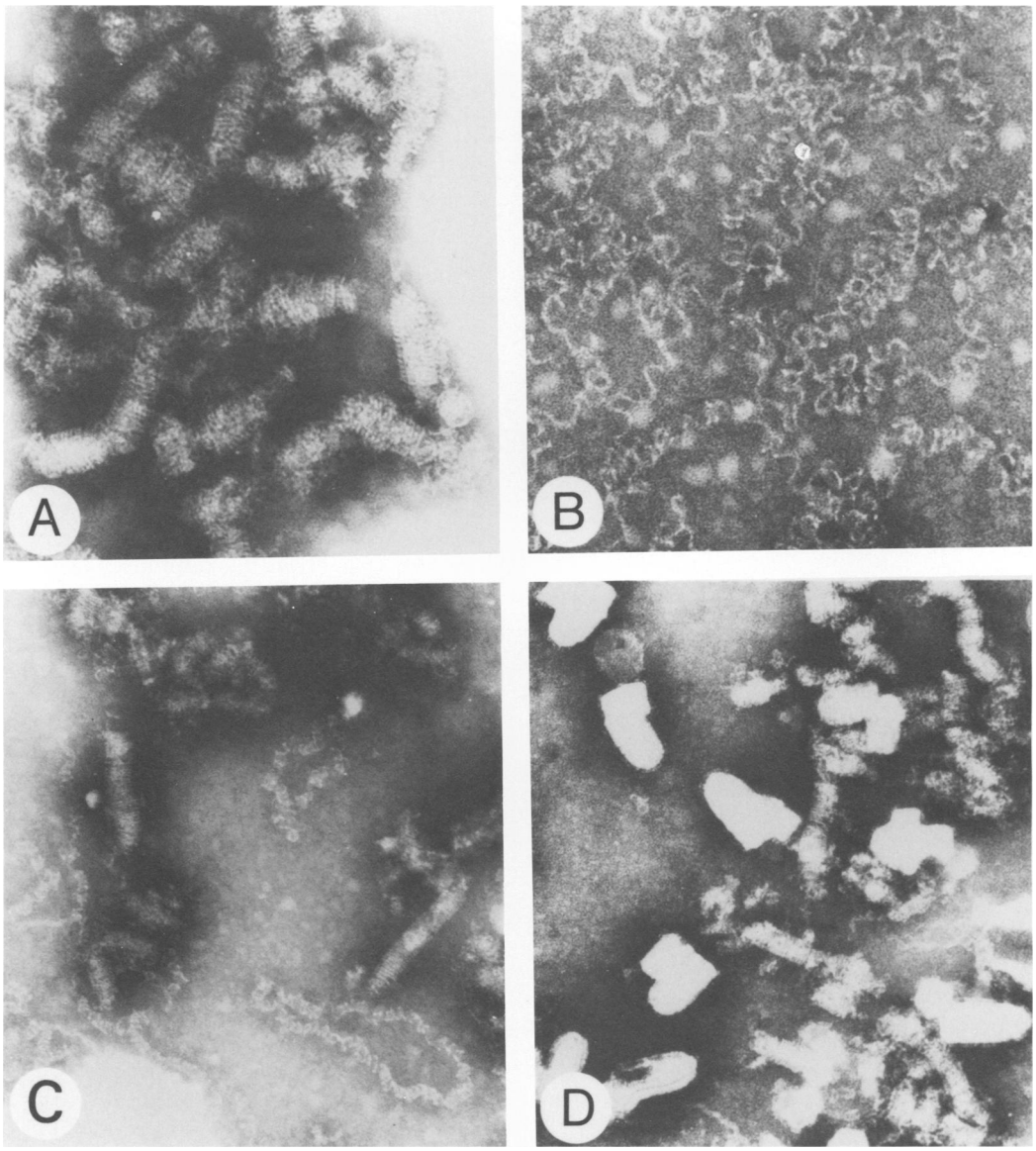

FIG. 1. *Electron micrographs of VSV extracted with 60 mM octylglucoside (A) and with 60 mM octylglucoside plus 0.5 M NaCl (B). Control micrographs show a mixture of VSV skeletons, from the sample shown in A, with nucleocapsids (C) and a mixture of skeletons with intact VSV (D). Magnifications were ×144,000 (A and B) and × 93,000 (C and D). VSV that was used in the experiments described here was grown on monolayer cultures of BHK-21 cells and purified by a two-step procedure involving rate-zonal followed by equilibrium ultracentrifugation, as previously described by Hunt and Wagner (5). Detergent extraction of purified virus suspensions was carried out as described in the text. Insoluble material was collected from detergent extracts by centrifugation in a Beckman SW50.1 rotor. Extraction solutions were layered on top of a 1-ml glycerol pad in 5-ml nitrocellulose SW50.1 tubes and centrifuged at 40,000 rpm (150,000 × g) for 90 min at 4°C in a Beckman L5-50 preparative ultracentrifuge. After centrifugation, the clear supernatant solutions were aspirated and the pelleted material was gently suspended in 0.5 ml of 0.01 M Tris-hydrochloride buffer. Samples to be examined in the electron microscope were first dialyzed overnight against 0.01 M Tris-hydrochloride buffer and adjusted to a concentration of 0.1 mg of viral protein per ml as determined by the method of Lowry et al. (6). Dialyzed samples were applied directly to Formvar-carbon-coated 300 mesh copper grids for 1 min and blotted dry. Grids were stained for 1.5 min with 2% phosphotungistic acid (pH 7.0), blotted, and examined in a JEOL 100c transmission electron microscope.*

TABLE 1. *Dimensions of VSV and VSV skeletons*[a]

Parameter	Intact VSV	Skeletons
Length	180.6 ± 10.2 nm	204.4 ± 6.0 nm
Diameter	67.0 ± 2.9 nm	51.7 ± 3.0 nm
Striations (spacing)	5.0 ± 0.65 nm	5.7 ± 0.75 nm[b]

[a] The dimensions of VSV and VSV skeletons were determined from measurements made on positive enlargements of electron microscope negatives. Results are reported as the mean value ± one standard deviation for at least 20 measurements of separate particles in representative microscopic fields.

[b] All measurements were made on complete skeletons lacking gaps between striations.

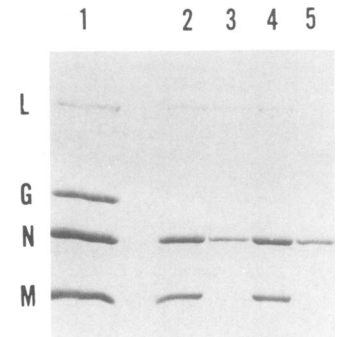

FIG. 2. *Sodium dodecyl sulfate-polyacrylamide gel analysis of VSV (lane 1) and insoluble products that result from extracting VSV with 60 mM octylglucoside (lane 2), 60 mM octylglucoside plus 0.5 M NaCl (lane 3), 1% Triton X-100 (lane 4), and 1% Triton X-100 plus 0.5 M NaCl (lane 5). The positions of the VSV L, G, N, and M proteins are indicated. Samples of VSV and VSV subviral particles to be analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were solubilized at a concentration of 1 to 2 mg of protein per ml by boiling for 2 min in 0.01 M Tris-hydrochloride buffer containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol. Samples (50 to 70 μl) of boiled preparations were mixed with 20 μl of glycerol containing 1% bromophenol blue tracking dye and subjected to electrophoresis on 7.5% polyacrylamide slab gels. Electrophoresis, fixing, staining, and destaining of the gels were carried out as described by Nagpal and Brown (10). Stained gels were photographed on Kodak Panatomic X film through a Telesar Red (R2) filter.*

useful experimental objects for the study of VSV structure. Production of VSV skeletons by detergent extraction in the absence of salt rationalizes the observation that these suspensions remain opaque, like untreated virus suspensions. Since skeletons have approximately the same shape as intact virus, they should scatter light in the same way.

The studies described here raise, but do not resolve, the issue of how the VSV M protein interacts with the nucleocapsid to produce compact skeleton structures. Skeletons such as those shown in Fig. 1A contain a large amount of M protein, but one cannot tell where it is located in the overall complex. Studies with intact VSV, however, have shown clearly that the M protein exists entirely within the virus membrane (7, 9, 14). Furthermore, recent studies have suggested that the M protein may lie close to the inner surface of the viral membrane. For example, the M, but not the N, protein can be cross-linked with dimethylsuberimidate to membrane phosphatidylethanolamine (12). Similarly, the M protein can be cross-linked to viral phospholipids by photoactivatable nitrenes, such as tartryldiazide and dithiobisphenylazide, but N protein cannot (20). These results are supported by the observation that purified M protein will bind to phospholipid vesicles (liposomes) containing negatively charged phospholipids (J. Zakowski, W. Petri, and R. Wagner, personal communication). Although the M protein must be found in reasonably close proximity to the VSV envelope, it is also clear from the results reported here that the M protein has a strong affinity for the viral nucleocapsid. Removal of the viral membrane with detergent at a low ionic strength yields skeletons in which M protein and nucleocapsids remain tightly associated. The experimental results emphasizing interaction of M protein with the viral membrane and those emphasizing interaction with the nucleocapsid can both be accomodated by assuming that, in native VSV, the M protein is found in the space between the membrane and the coiled nucleocapsid as suggested in current proposed structures for the virus (16).

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