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In Vitro Reassembly of Vesicular Stomatitis Virus Skeletons

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Vesicular stomatitis virus (VSV) has been disrupted with nonionic detergent plus 0.5 M NaCl under conditions which result in solubilization of the viral glycoprotein (G), matrix protein (M), and lipids, leaving the nucleocapsid in a highly extended state. Dialysis of these suspensions to remove NaCl was found to result in reassociation of nucleocapsids with M protein. Reassociated structures were highly condensed and similar in appearance to "native" VSV skeletons produced by extraction of virions with detergent at low ionic strength. For instance, electron microscopic analysis revealed that, like "native" skeletons, "reassembled" skeletons were cylindrical in shape, with diameters in the range of 51.0 to 55.0 nm and cross-striations spaced approximately 6.0 nm apart along the length of the structure. Like native skeletons, reassembled skeletons were found by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to contain the viral N and M proteins, but they lacked the glycoprotein entirely. Both native and reassembled skeletons were found to be capable of in vitro RNA-dependent RNA synthesis (transcription). In vitro skeleton assembly required the presence of M protein and nucleocapsids. No skeleton-like structures were formed by dialysis of nucleocapsids in the absence of M protein or of M protein in the absence of nucleocapsids. These results provide strong support for the view that the VSV M protein plays a functional role in condensing the viral nucleocapsid in vitro and raise the possibility that it may play a similar role in vivo.

Recent experiments in our laboratory have emphasized the fact that NaCl can profoundly affect the disruption of vesicular stomatitis virus (VSV) by nonionic detergents (14). Extraction of native virions with 60 mM octylglucoside or 1% Triton X-100 at low ionic strength results in solubilization of the viral glycoprotein (G) and lipids, leaving insoluble structures called "skeletons" (1, 12). Skeletons contain the viral RNA complexed with the N and M proteins in highly condensed and very regular structures that have the same overall cylindrical shape and striated appearance as the native virus. Similar structures are produced by extraction with digitonin (18) or with Tween 80 plus diethylether (2). In contrast, detergent extraction in the presence of 0.5 M NaCl results in solubilization of the G protein, the M protein, and lipids, liberating the viral nucleocapsid in an irregular and highly extended state. One molar KCl was found to have a similar effect on the extraction of Sendai virus with 2% Triton X-100 (15).

The dramatic effect of NaCl on the detergent-induced disruption of VSV has motivated us to ask whether its removal from detergent-high salt–solubilized virus might promote some degree of reassociation of viral components. Intact vesicular stomatitis virions were, therefore, solubilized in nonionic detergent containing 0.5 M NaCl and then dialyzed against a low-ionic-strength buffer to remove NaCl. The results of this basic experiment, described below, show that dialysis is accompanied by the reassembly of VSV nucleocapsids and M protein to form condensed structures quite similar to "native" VSV skeletons.

MATERIALS AND METHODS

Cell and virus growth. All experiments were performed with the Mudd-Summers strain of VSV (Indiana), which was grown on monolayer cultures of BHK-21 cells. Cells were propagated at 37°C in 150-cm^2 plastic tissue culture flasks containing 40 ml of Dulbecco modified minimal essential medium supplemented with 10% calf serum, 10% tryptose phosphate broth, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml. Virus stocks were grown at a low multiplicity of infection as previously described (5) and were shown by rate-velocity ultracentrifugation to be free from defective interfering particles. Viral proteins were radioactively labeled by including 5 μCi of L-[^{3}H]leucine (60 Ci/mmol; Amersham Corp.) per ml in the virus growth medium. VSV was purified from the infected cell medium by a two-step procedure involving rate-zonal followed by equilibrium ultracentrifugation, essentially as described by Hunt and Wagner (9). The purified virus preparations were judged to be free from cellular contamination by sodium dodecyl sulfate (SDS)-polyacrylamide gel analysis which revealed only the five VSV proteins (N, NS, M, G, and

L); no contaminating cellular proteins could be detected.

Disruption of VSV. Freshly purified VSV to be extracted with detergent was gently suspended in 0.01 M Tris-hydrochloride buffer, pH 7.4, and centrifuged for 10 min at 1,000 × *g* to remove virus aggregates. The supernatant virus suspension was then extracted with either octylglucoside (1-*O*-*n*-octyl-β-D-glucopyranoside; Calbiochem, La Jolla, Calif.) or Triton X-100 in the presence of 0.5 M NaCl. Octylglucoside extraction was carried out by adjusting the virus suspension to 60 mM octylglucoside, 0.5 M NaCl, 5 mM dithiothreitol, 10% glycerol, and 10 mM Tris-hydrochloride buffer, pH 7.8 (octylglucoside–0.5 M NaCl dissociation medium) at 0°C, and a concentration of 0.1 to 0.2 mg of viral protein per ml. Similar conditions were employed for Triton X-100 extraction except that 1% Triton X-100 was substituted for 60 mM octylglucoside. In both cases virus suspensions were thoroughly but gently mixed with detergent plus NaCl and allowed to stand on ice (0°C) for 30 min before further operations were performed. The initially turbid virus suspensions were clarified immediately upon addition of detergent plus NaCl, and the suspensions remained clear during the 30-min period allowed for disruption. Detergent extraction of VSV in the absence of NaCl was carried out as described previously (14).

Reassembly conditions. VSV components were allowed to reassociate during dialysis against 0.01 M Tris-hydrochloride (pH 7.4)–5 mM dithiothreitol (TB). Four-milliliter samples of VSV disrupted in detergent plus 0.5 M NaCl as described above were dialyzed against TB for 16 h at 4°C and for an additional 12 h at 25°C. If dialyzed solutions were not analyzed immediately, they were stored on ice for a maximum of 4 h.

Ultracentrifugation. Insoluble material was pelleted from suspensions of dissociated VSV or from reassembly mixtures by ultracentrifugation in 5-ml Beckman SW50.1 nitrocellulose tubes. Four-milliliter samples of dissociated or reassembled viral components were layered on top of a 0.5-ml "cushion" of glycerol and were centrifuged at 38,000 rpm (130,000 × *g*) for 2 h at 4°C. The fluid above and including the interface of the glycerol pad was aspirated while the remainder of the pad, containing the pelleted material, was mixed with 0.5 ml of TB and dialyzed overnight at 4°C to remove glycerol. Dialyzed samples were used directly for electron microscopic and transcription analyses or were lyophilized prior to SDS-polyacrylamide gel electrophoresis.

Both rate-velocity and sedimentation equilibrium sucrose gradient methods were employed to purify subviral structures from dissociated and reassociated VSV components. Rate-velocity analyses began with 0.5 ml of sample which was layered onto the top of 10 to 70% (wt/vol) linear sucrose gradients prepared in 5-ml Beckman SW50.1 nitrocellulose tubes. Stock sucrose solutions were prepared by dissolving ultrapure sucrose (Schwarz/Mann) in TB. Gradients were centrifuged at 38,000 rpm for 90 min at 4°C in an SW50.1 rotor. After centrifugation, the gradients were fractionated from the bottom by use of a peristaltic pump and an LKB-7000 fraction collector. The radioactivity present in each fraction was determined by dissolving a 20-μl sample in 0.5 ml of NCS tissue solubilizer (Amersham Corp.) containing 10 ml of toluene-based scintillation cocktail (Research Products International, Grove Village, Ill.) and counting in a Packard model 3320 liquid scintillation spectrometer. Gradient fractions to be analyzed further were dialyzed against TB overnight at 4°C to remove sucrose and then were stained directly for electron microscopy or lyophilized for SDS-polyacrylamide gel analysis. Equilibrium ultracentrifugation was carried out by the same method described for rate-velocity centrifugation except that centrifugation was for 16 h rather than 90 min. The density of each gradient fraction was determined by weighing a 100-μl sample on a Mettler H18 analytical balance.

In vitro RNA-dependent RNA synthesis and polyacrylamide gel analysis of RNA. Native and reassembled skeletons were tested for the presence of endogenous RNA-dependent RNA polymerase activity by the assay ordinarily employed for intact VSV (4). Assay mixtures contained 0.14 M NaCl, 0.2% Triton X-100, 7.5 mM $MgCl_2$, 0.01 M Tris-hydrochloride, pH 7.9, 1 mM dithiothreitol, 0.3 mM ATP, GTP, and CTP, and 0.03 mM [α-^{32}P]UTP (0.45 Ci/mmol) plus 25 to 35 μg of viral protein in a total volume of 30 μl. Incubations for up to 3 h were carried out at 31°C, after which 5-μl samples of the reaction mixture were precipitated in 1 ml of 5% trichloroacetic acid. Precipitated RNA was collected by filtration on 24-mm filters (Millipore Corp.), dried, and counted at an efficiency of 65% in toluene-based liquid scintillation cocktail. Results were expressed as nanomoles of [^{32}P]UMP incorporated per minute per milligram of protein.

Other methods. Electron microscopic analysis was carried out with subviral structures negatively stained with 2% phosphotungstic acid (pH 7.0) as described previously (14). Particle measurements were made on positive enlargements of electron microscope negatives. SDS-polyacrylamide gel electrophoresis and gel staining with Coomassie blue were performed on 2-mm-thick slab gels as described by Nagpal and Brown (13). Seven-millimeter lanes were loaded with samples containing 50 to 100 μg of viral protein. All gels contained a lane, labeled "std," for electrophoresis of solubilized VSV, and positions of the five VSV proteins (L, G, N, NS, and M) are indicated. All protein concentrations were determined by the Lowry method (11).

RESULTS

Disruption of VSV in nonionic detergent plus NaCl. Vesicular stomatitis virions were disrupted in nonionic detergent plus 0.5 M NaCl under conditions shown previously to solubilize the viral envelope components and release the nucleocapsid in an uncondensed form (14). The extent of detergent-NaCl–induced virus disassembly was monitored by electron microscopy, by sedimentation velocity ultracentrifugation, and by SDS-polyacrylamide gel electrophoresis. Figure 1A shows a negatively stained preparation of insoluble structures pelleted from VSV disrupted with 60 mM octylglucoside–0.5 M NaCl by centrifugation for 2 h at 130,000 × *g* as described in Materials and Methods. Pelleted material was found to contain only extended viral nucleocapsids (6); no intact VSV or condensed VSV skeletons could be detected. It

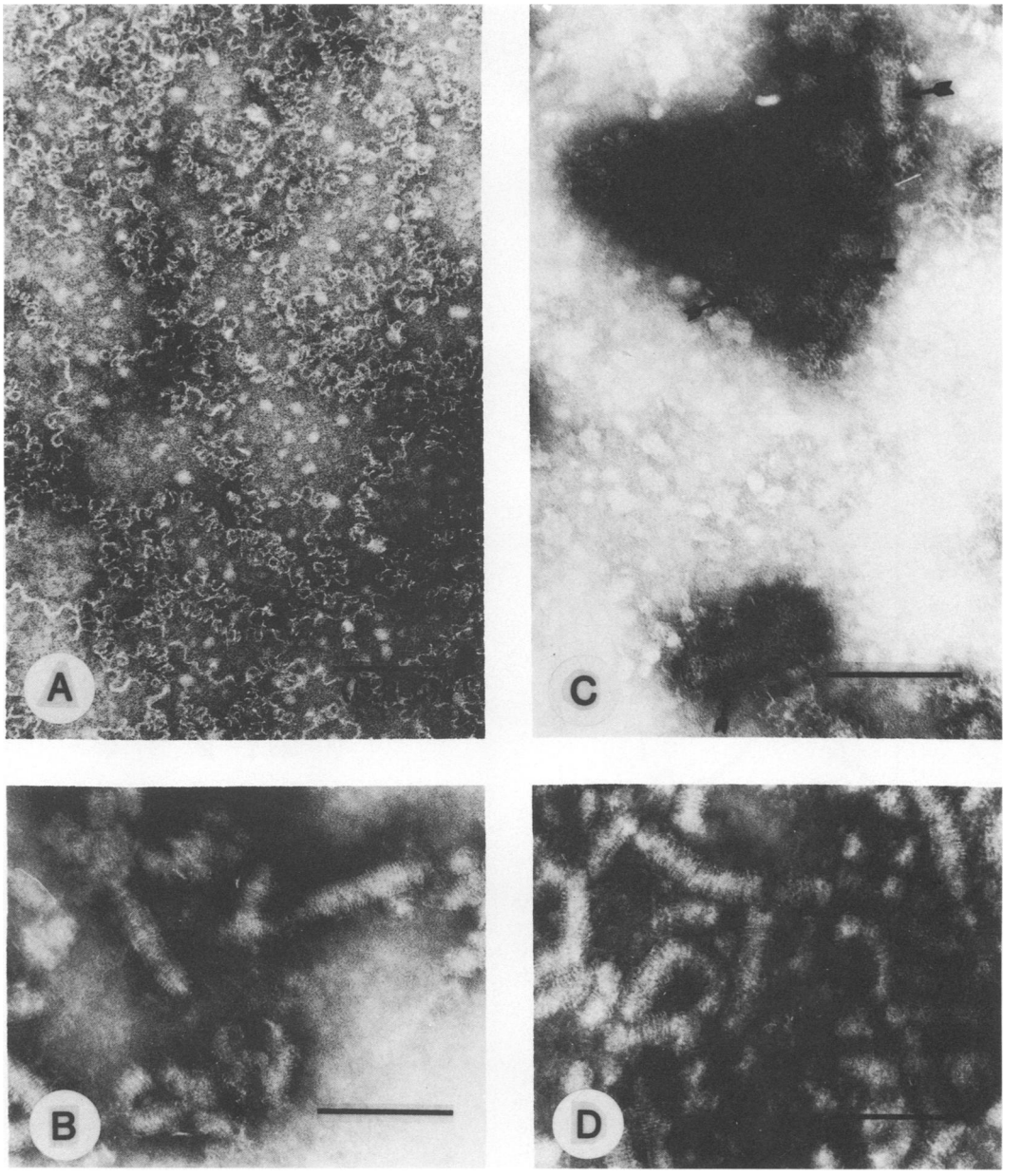

FIG. 1. Electron micrographs of detergent-extracted VSV and reassembled VSV skeletons. (A) Nucleocapsids isolated by centrifugation of solutions produced by extracting VSV with 60 mM octylglucoside plus 0.5 M NaCl. (B) Skeletons isolated by centrifugation of suspensions produced by extracting VSV with 60 mM octylglucoside. (C) Reassembled skeletons (arrows) identified by direct observation of the suspension resulting from dialysis of VSV solubilized with 60 mM octylglucoside–0.5 M NaCl. (D) Reassembled skeletons isolated by centrifugation of the suspension resulting from dialysis of VSV solubilized with 60 mM octylglucoside–0.5 M NaCl. (A) ×105,000; (B, C, and D) ×125,000. Bar = 0.2 μm.

must be, therefore, that all or nearly all intact virus particles were disassembled.

Figure 2A shows the results obtained when VSV, grown in the presence of [^{3}H]leucine, was disrupted in 60 mM octylglucoside plus 0.5 M NaCl and analyzed by rate-velocity sedimentation on 10 to 70% sucrose gradients. Two bands of ^{3}H-labeled viral proteins were observed, one (band II) at the top of the gradient where soluble proteins should occur and the other (band I)

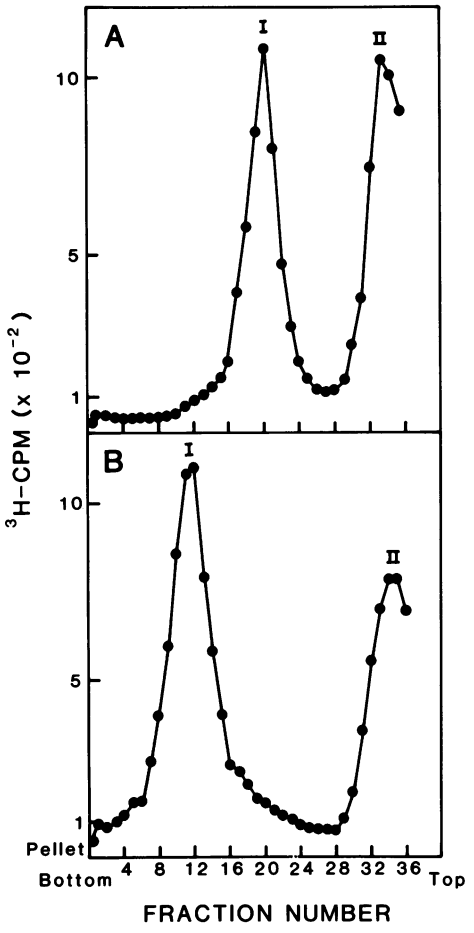

FIG. 2. Rate-velocity ultracentrifugation of VSV solubilized with 60 mM octylglucoside–0.5 M NaCl before (A) and after (B) dialysis against TB. VSV employed in these experiments was radioactively labeled by growth in the presence of [^{3}H]leucine.

farther down the gradient. Electron microscopic analysis of band I demonstrated that it contained extended viral nucleocapsids indistinguishable from those shown in Fig. 1A. SDS-polyacrylamide gel analysis, as shown in Fig. 3, demonstrated that band I contained predominantly N protein (lane 2) whereas band II contained both G and M proteins (lane 1). Virtually identical results of both electron microscopic and sucrose gradient analyses were obtained when 1% Triton X-100 was substituted for 60 mM octylglucoside in the virus dissociation medium. We conclude that disruption of VSV in nonionic detergent plus 0.5 M NaCl results in solubilization of the G and M proteins and release of the viral nucleocapsid in an extended state.

Reassociation of VSV components: electron microscopic analysis. Reassociation of viral components was assayed after vesicular stomatitis virions were disrupted in nonionic detergent containing 0.5 M NaCl and then dialyzed against TB. Dialysis was expected to remove detergent and NaCl in the case of virus disrupted in octylglucoside–0.5 M NaCl and NaCl only in the case of Triton X-100–0.5 M NaCl disruption (8, 17). Dialysis was found to promote reappearance of turbidity in initially clear solutions of viral components. Turbidity increased gradually during the first 12 to 18 h of dialysis and did not change thereafter. Direct electron microscopic analysis of dialyzed suspensions revealed the presence of condensed structures, as shown in Fig. 1C. Extended nucleocapsids were very rare or absent altogether. Condensed structures all appeared to be made up of a fine filament or thread. In some cases the thread or threads appeared to be clumped in an irregular way to form roughly spherical particles ("tangled clumps"), as shown in the upper left of Fig. 1C. Quite often, however, condensed particles were cylindrical in shape with cross-striations over the central portion of the overall structure and irregularly packaged threads at the ends (arrows in Fig. 1C). The cylinder diameter and the

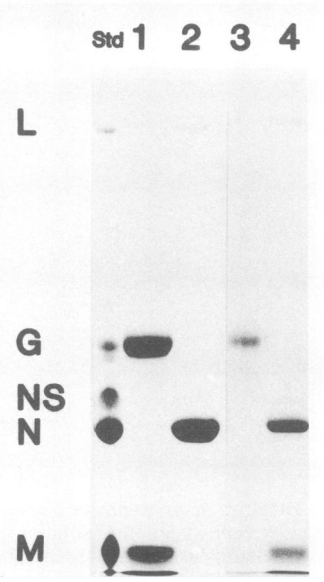

FIG. 3. SDS-polyacrylamide gel analysis of subviral structures separated by rate-velocity ultracentrifugation of VSV solubilized with 60 mM octylglucoside–0.5 M NaCl before (lanes 1 and 2) and after (lanes 3 and 4) dialysis against TB. Lane 1: band II from Fig. 2A. Lane 2: band I from Fig. 2A. Lane 3: band II from Fig. 2B. Lane 4: band I from Fig. 2B. The gel was stained with Coomassie blue, and the positions of standard VSV protein are indicated.

spacing between cross-striations were found to be similar to those observed for "native" VSV skeletons, as shown in Table 1. The length of the cross-striated region was variable but never greater than the length of native skeletons. The morphological similarity of native and reassembled skeletons was emphasized by electron microscopic analysis of insoluble material pelleted from reassembly mixtures by ultracentrifugation at 130,000 × *g* for 2 h. Reassembled skeletons harvested in this way were indistinguishable from native skeletons, as shown in Fig. 1D and B.

Control experiments showed that VSV nucleocapsids and soluble components must be present together for striated skeletons to form during dialysis. These experiments involved disassembly of native virions in 60 mM octylglucoside plus 0.5 M NaCl as described above and then centrifugation to remove the nucleocapsids from solution. The supernatant containing solubilized viral G protein, M protein, and lipids (soluble components) was then dialyzed against TB under the same conditions employed for reassembly of viral skeletons. Similarly, the isolated nucleocapsids were resuspended at a concentration of 0.1 mg of protein per ml in octylglucoside–0.5 M NaCl dissociation medium and dialyzed against TB. Electron microscopic analysis of the two dialyzed fractions revealed that neither contained skeletons or skeleton-like structures. Isolated nucleocapsids gave images similar to Fig. 1A both before and after dialysis. Dialysis of soluble components resulted in formation of a precipitate which pelleted after centrifugation at 600 × *g* for 5 min. SDS-polyacrylamide gel analysis showed that the precipitate contained M protein only (data not shown); the glycoprotein remained in the supernatant.

Protein composition of reassembled skeletons. The protein composition of reassembled skeletons was determined by SDS-polyacrylamide gel analysis of structures purified from reassembly mixtures by sucrose density gradient ultracentrifugation. Preparation of reassembled skeletons began with VSV grown in the presence of [^{3}H]leucine. Purified virions were disrupted in nonionic detergent plus 0.5 M NaCl, and the resulting solutions were dialyzed to reassemble skeletons as described above. Reassembled skeletons were purified from the dialyzed suspensions by rate-velocity ultracentrifugation in one set of experiments and by sedimentation equilibrium in another. Figure 2B shows the results of rate-velocity purification performed with skeletons reassembled after disruption of VSV with 60 mM octylglucoside plus 0.5 M NaCl. ^{3}H-labeled viral components were found to sediment in two bands, one (band II) near the top of the gradient and the other (band I) centered at fraction 12. Electron microscopic analysis revealed the presence of reassembled skeletons in band I, but not in band II (micrographs not shown). SDS-polyacrylamide gel analysis of band I from Fig. 2B (reassembled skeletons) demonstrated that it contained the viral M and N proteins, as shown in Fig. 3, lane 4. No G protein was detected in reassembled skeleton preparations; G protein was found in band II at the top of the gradient (see Fig. 3, lane 3). Results essentially identical to those shown in Fig. 2 and 3 were obtained when 1% Triton X-100 was substituted for 60 mM octylglucoside in the virus dissociation medium.

TABLE 1. Dimensions of native and reassembled VSV skeletons[a]

Skeletons	Length (nm)	Diam (nm)	Spacing of striations (nm)
Native	204.4 ± 6.00	51.7 ± 3.00	5.7 ± 0.75
Reassembled	Variable[b]	53.7 ± 2.70	6.2 ± 0.32

[a] Results are reported as the mean value ± 1 standard deviation for at least 20 measurements of representative particles. Data for native skeletons are from Newcomb and Brown (14).

[b] The length of the cylindrical, striated portion of reassembled skeletons was variable but never greater than 200.0 nm.

When dialyzed reassociation mixtures were centrifuged to equilibrium, two bands of ^{3}H-labeled viral components were observed, one (band I) with a buoyant density of approximately 1.26 g/cm^3 and the other (band II) near the top of the gradient. Electron microscopic analysis demonstrated the presence of reassembled skeletons in band I; none were found in band II. SDS-polyacrylamide gel analysis of band I material showed that M and N were the predominant protein components present (see Fig. 4, lanes 1 and 3). As in the case of reassembled skeletons purified by rate-velocity centrifugation, little or no G protein could be detected in reassembled skeletons purified by equilibrium sedimentation. Instead, G protein was found in band II at the top of equilibrium gradients, as shown in Fig. 4, lanes 2 and 4. The identity of the disrupting detergent did not substantially affect either the buoyant density or the protein composition (compare lanes 1 and 3 in Fig. 4) of reassembled skeletons. Both parameters were virtually the same in skeletons reassembled from virions disrupted with octylglucoside–NaCl and with Triton X-100–NaCl.

RNA-dependent RNA polymerase activity. In vitro assays showed that both native and reassembled skeletons contained endogenous RNA-dependent RNA polymerase activity. Their specific polymerase activities were found to be

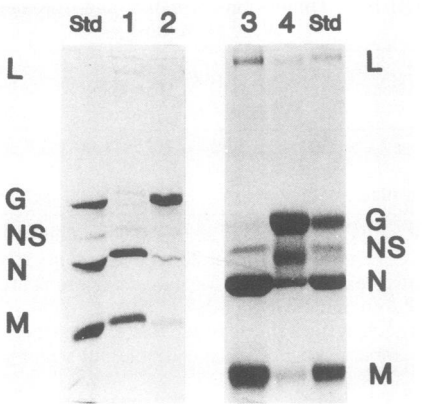

FIG. 4. SDS-polyacrylamide gel analysis of reassembled skeletons and soluble components separated by sedimentation equilibrium ultracentrifugation. Lane 1: skeletons (band I) reassembled from VSV solubilized with 60 mM octylglucoside–0.5 M NaCl. Lane 2: soluble components (band II) remaining after reassembly of skeletons from VSV solubilized with 60 mM octylglucoside–0.5 M NaCl. Lane 3: skeletons (band I) reassembled from VSV solubilized with 1% Triton X-100–0.5 M NaCl. Lane 4: soluble components (band II) remaining after reassembly of skeletons from VSV solubilized with 1% Triton X-100–0.5 M NaCl. Gels were stained with Coomassie blue, and the positions of standard VSV proteins are indicated.

comparable to the activity observed for VSV nucleocapsids, as shown in Table 2, but substantially lower than the specific activity of intact virions assayed under the same conditions. Polyacrylamide gel analysis revealed an overall pattern of similarity in the RNA species synthesized by reassembled skeletons, native skeletons, and native VSV. In all cases "leader" RNA (3) and many higher-molecular-weight RNA species were synthesized (data not shown).

DISCUSSION

The results reported here demonstrate the formation of VSV skeletons in vitro. Skeleton reassembly took place during overnight dialysis of solutions produced by dissolving native vesicular stomatitis virions in nonionic detergent containing 0.5 M NaCl. The skeletons which reassembled under these conditions were found to be virtually indistinguishable from "native" VSV skeletons isolated by extracting intact virus with nonionic detergent at low ionic strength (14). For example, both native and reassembled skeletons were found to be cross-striated cylinders, with diameters of 51 to 55 nm and striations spaced approximately 6 nm apart. Both native and reassembled skeletons contained the viral N protein, M protein, and RNA, but both lacked the G protein entirely. Like native skeletons, reassembled skeletons were found to be capable of in vitro RNA-dependent RNA synthesis. There can be little question, therefore, that reassembled skeletons are faithful replicas of their native counterparts.

It is most likely that removal of salt, not removal of detergent, was responsible for promoting skeleton formation in vitro. Skeletons formed abundantly during dialysis of virus solubilized with octylglucoside–NaCl when both detergent and NaCl were removed. They also formed normally from virus solubilized with Triton X-100–NaCl when only NaCl was removed. The presence of detergent, therefore, did not appear to affect skeleton formation in vitro, but the presence of NaCl clearly did. Salt was found to have a similar effect on the ability of Semliki Forest virus glycoproteins to associate with Semliki Forest virus nucleocapsids in vitro. Glycoprotein-nucleocapsid interaction was observed in 24 mM octylglucoside–10 mM sodium phosphate buffer, pH 6.8, but not in the same solution containing 0.1 M NaCl at pH 8.0 (7).

Although mature skeletons were the predominant structures formed during dialysis of solubilized VSV, other related structures were also observed at lower frequency. These included the "tangled clumps" described above and tangled clumps containing central cross-striations. Tangled clumps appeared to consist of a fine thread wound in an irregular way to form a roughly spherical particle. The thread diameter was smaller than the diameter of VSV nucleocapsids, and no nucleocapsids were found to protrude from tangled clumps. Cross-striated tan-

TABLE 2. RNA-dependent RNA synthesis by native skeletons, reassembled skeletons, and nucleocapsids[a]

Preparation	Specific polymerase activity[b]	% of native VSV
Native VSV	1.78	100
Native VSV skeletons	0.45	25
Nucleocapsids	0.44	25
Reassembled skeletons (Triton X-100)	0.38	21
Reassembled skeletons (octylglucoside)	0.89	50

[a] Native skeletons and nucleocapsids were prepared by disruption of VSV in 60 mM octylglucoside in the absence of salt and in the presence of 0.5 M NaCl, respectively, as described previously (14). Reassembled skeletons were prepared by dialysis of VSV solubilized with Triton X-100–NaCl or octylglucoside–NaCl as described in Materials and Methods.

[b] Expressed as nanomoles of [^{32}P]UMP incorporated per minute per milligram of protein.

gled clumps were similar in appearance to tangled clumps except that they contained a central cylindrical region with regularly spaced striations and tangled clumplike material at both ends. The central cylindrical region was of various lengths in different particles, but its diameter and striation spacing were the same as those found in mature skeletons. Particles of this type can be seen in Fig. 1C (arrows). We presume that the minor components (tangled clumps and cross-striated tangled clumps) found in reassembly mixtures are intermediates in the skeleton self-assembly process. Their structural relationship to mature skeletons suggests that skeleton reassembly in vitro took place in the following way. Nucleocapsids and M protein aggregated to form tangled clumps in which condensed nucleocapsids were surrounded by a fine thread composed of M protein. Cross-striations were then formed by subsequent internal rearrangements in the tangled clump structure. This proposed pathway for skeleton reassembly is shown schematically in Fig. 5. Further experimental studies will be required to substantiate its role in skeleton reassembly.

The results described in this paper indicate that M protein must have a very strong affinity for VSV nucleocapsids. This can be appreciated from the fact that very few, if any, free nucleocapsids remained after dialysis of virions solubilized with detergent–0.5 M NaCl; virtually all were complexed with M protein in condensed structures (see Fig. 2B). Similarly, nearly all M protein became associated with nucleocapsids. Very little remained in the soluble fraction, as shown in Fig. 3, lane 3, or Fig. 4, lanes 2 and 4. This contrasts to the behavior of the glycoprotein which was present during dialysis of solubilized VSV, but was not incorporated into reassembled skeletons. The interaction of nucleocapsids with M protein must, therefore, be very strong and at least somewhat specific.

The abundant formation of VSV skeletons in vitro raises the possibility that similar structures may be formed in vivo. Calculations based on the data of Simonsen et al. (16) show that the molar concentrations of nucleocapsids and M protein in VSV-infected HeLa cells are comparable to or greater than their concentrations in the reassembly experiments described here. Depending on the values assumed for the cell volume and the time after infection, the concentration of negative-strand nucleocapsids in infected cells falls in the range of 2×10^{-9} to 4×10^{-9} M and that of M protein is approximately 2×10^{-6} to 5×10^{-6} M. The concentrations employed for in vitro skeleton reassembly in our experiments were 1×10^{-9} to 2×10^{-9} M for nucleocapsids and 1.2×10^{-6} to 2.4×10^{-6} M for M protein. The concentrations of nucleocapsids and M protein are, therefore, high enough to support skeleton formation in infected cells.

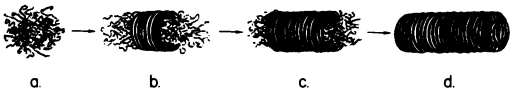

FIG. 5. Proposed pathway for in vitro reassembly of VSV skeletons. (a) Tangled clumps, (b and c) cross-striated tangled clumps, and (d) reassembled skeletons.

Experimental results of Knipe et al. (10) also suggest that M protein may associate with VSV nucleocapsids in vivo. These authors examined CHO cells infected with a strain of VSV, tsM601, which carries a temperature-sensitive lesion in the N protein. An unusually low level of VSV nucleocapsids accumulated in cells infected with tsM601 at the nonpermissive temperature (39°C); N protein was rapidly degraded, and little full-length (42S) viral RNA was synthesized. In contrast, G protein was made in normal amounts and it migrated normally to the plasma membrane. M protein was also synthesized in normal amounts, but it accumulated in soluble form in the cytoplasm rather than becoming attached to membranes as it does in cells infected with wild-type VSV. This is the result that would be expected if M protein could associate with membranes only after it had bound to nucleocapsids.

ACKNOWLEDGMENTS

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