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Structural Proteins of Vesicular Stomatitis Viruses

ROBERT R. WAGNER, TERRY A. SCHNAITMAN, AND RUTH M. SNYDER

Department of Microbiology, The University of Virginia School of Medicine, Charlottesville, Virginia 22901

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Three major and three minor structural proteins were identified by polyacrylamide gel electrophoresis of purified infectious virions of the Indiana serotype of vesicular stomatitis (VS) virus disrupted with acetic acid, 0.5 M urea, sodium dodecyl sulfate (SDS), and 2-mercaptoethanol. Molecular weights of the six virion proteins were estimated by comparative electrophoretic migration of known marker proteins in the presence of SDS. The following values were obtained: major proteins P6 $\cong$ 34,500, P5 $\cong$ 59,500, and P4 $\cong$ 81,500; minor proteins P3 $\cong$ 140,000, P2 $\cong$ 186,000, and P1 $\cong$ 275,000. P1 did not disaggregate in 8 M urea, but P2 and P3 did. The possibility that P1 is an uncleaved large polypeptide chain could not be ruled out. Six identical protein components were dissociated from Indiana VS virions grown in chick and mouse cells; no cellular proteins could be detected in purified virions. Of six proteins identified in virions of the New Jersey serotype, only the smallest protein (P6) could be distinguished from any of the six proteins of the Indiana serotype on the basis of migration in SDS gels. The defective T particles of Indiana VS virus contained the same six proteins in essentially the same proportions as those of the infectious B virions. Only P6 and P5 could be cleanly separated by preparative gel electrophoresis.

Vesicular stomatitis (VS) viruses are morphologically distinctive and structurally complex (2, 19). VS virions form by budding from the cell surface and appear to be encased in an envelope which is anatomically contiguous with the cytoplasmic membrane (17; J. W. Greenawalt and R. R. Wagner, *unpublished data*). Cartwright and Pearce (3) presented evidence for the presence of specific cellular antigens in the envelope derived from the cell species in which the virions were grown. There are two distinct antigenic types of VS virus, designated Indiana and New Jersey serotypes (16), and perhaps a third type. Three antigenic components have been identified in virions of the Indiana serotype (1). Released infectious virions are characteristically bullet-shaped (B particles), planar at one end and hemispheric at the other. A preponderance of truncated, noninfectious forms (T particles) are produced by cells infected at high multiplicity (8). These T particles are identical in ultrastructure to the B virions but are only one-third their length and contain only a one-third piece of ribonucleic acid (RNA; references 7 and 17).

The experiments reported herein were undertaken to characterize and compare the structural proteins of the two major serotypes of VS virus, as well as the structural proteins of the infectious B and the defective T forms grown in cells of different animal species. Our original hypothesis, that significant differences would be found among the various forms of VS virus, turned out to be incorrect.

MATERIALS AND METHODS

Viruses and cells. The origin and methods of cultivation of the Indiana (VS_{Ind}) and New Jersey (VS_{NJ}) strains of vesicular stomatitis virus have been described (8, 24). Both strains have been cloned periodically by plaque purification. Virus was grown in bulk by infecting confluent monolayer cultures of $\sim 2 \times 10^7$ chick embryo (CE) cells in milk dilution bottles (40 cm^2 surface) or $\sim 1.8 \times 10^7$ mouse L cells in Falcon plastic flasks (75 cm^2 surface). Growth medium for CE cells consisted of 0.5% lactalbumin hydrolysate; for L cells, medium 199 in Earle's saline solution was used. Each was supplemented with 5 to 10% calf serum. The media used for virus propagation and radioisotope labeling were Eagle's (BME) basal medium and minimal essential medium (MEM), both purchased from Grand Island Biological Co., Grand Island, N.Y. Plaque assays of VS viruses were performed as previously described (24).

Radioisotopes. ^3H-leucine (10.3 to 14.7 c/mmole), ^3H-tyrosine (28.2 to 43 c/mmole), and ^{14}C-amino acids of *Chlorella vulgaris* protein hydrolysate (54 mc/mAtom of carbon) were from Nuclear Chicago Corp., Des Plaines, Ill.

Labeling and partial purification of virus. In each experiment, 10 monolayer cultures of CE or L cells were infected at an input multiplicity of 5 to 50 plaque-forming units of VS virus. The inoculum was prepared

in a volume of 0.5 ml and adsorption was for 30 to 60 min at 37 C. For ^{3}H-protein labeling, the 10 cell cultures were each covered with 5 ml of special BME containing both ^{3}H-leucine (10 μc/ml) and ^{3}H-tyrosine (10 μc/ml) but without cold leucine, cold tyrosine, or serum. For ^{14}C-protein labeling, the 10 cell monolayers were each covered with 5 ml of complete BME diluted 1:50 in Earle's saline solution containing uniformly labeled ^{14}C-amino acids (1.13 μc/ml) but without serum. After incubation in the serum-free medium for 8 hr at 37 C, 5 ml of MEM with 2% calf serum was added to each culture. The media from 10 infected cultures were harvested at 17 hr, pooled, and centrifuged at 1,500 rev/min for 10 min to remove gross cellular debris. The 100-ml pools were then centrifuged twice for 1 hr at 30,000 rev/min. The final pellets were washed again and suspended in 1 ml of Earle's saline solution, and virus clumps were disaggregated by sonic oscillation for 30 sec at the 1-amp setting of a Raytheon sonic oscillator. Plaque counts of unconcentrated virus were 2×10^8 to 8×10^8, and plaque counts of the 100× concentrate were 2×10^{10} to 5×10^{10} when plated on CE or L cells.

As previously described (8), 1 ml of the 100× virus concentrate was then layered on a 0 to 40% linear sucrose gradient and centrifuged for 90 min at 18,000 rev/min in an SW 25.1 Spinco rotor. Easily visible and clearly separated light-scattering bands of B and T particles were collected by syringe and needle from the side of the tube. The separated B or T particles were repelleted at 40,000 rev/min for 90 min and suspended in 0.5 to 1.0 ml of Earle's saline solution. Some preparations were repurified by another cycle of sucrose gradient centrifugation; others were still further purified by equilibrium centrifugation at 38,000 rev/min for 72 hr in an SW50 rotor in a supporting medium of CsCl (starting specific gravity, 1.23 g/ml). Light-scattering bands were visible. Fractions of 0.2 ml each were collected from the bottom of each tube with the aid of a Beckman fractionating system and assayed for radioactivity and for density by weighing in 50-μliter pipettes. These procedures result in considerable distortion of virions and reduction in infectivity (8).

Protein content of purified and concentrated suspensions of virus was estimated by the method of Lowry et al. (11) with crystalline bovine serum albumin as a standard.

Dissolution of virion polypeptides. The method used was essentially that of Maizel et al. (12–15). One-tenth volume of glacial acetic acid was added to 100 to 200 μliters of purified B or T virus particles containing 2.5 to 3.5 mg of protein per ml. The acidified virus suspensions were then made 0.5 or 8 M with respect to urea and 1% with respect to sodium dodecyl sulfate (SDS). After incubation for 1 hr at 37 C, the suspension was dialyzed at room temperature for 16 to 18 hr against 200 to 400 ml of phosphate buffer (*p*H 7.2, 0.01 M) containing 0.1% SDS, 0.5 M urea, and 0.1% 2-mercaptoethanol. One-fourth volumes of 40% sucrose and 5 μliters of 1% bromophenol blue (aqueous) were added to the samples just prior to applying them to the gels.

Polyacrylamide gel electrophoresis. The most satisfactory separation of viral proteins was accomplished with gels composed of 7.5 or 5% acrylamide, 0.2% or 0.13% *N,N'*-bis-methylene acrylamide, 0.5 M urea, and 0.1% SDS in 0.1 M phosphate buffer, *p*H 7.2. Gels were polymerized with ammonium persulfate and *N,N,N',N'*-tetramethyl-ethylenediamine. The upper and lower reservoirs contained 0.1 M phosphate buffer and 0.1% SDS. Samples were layered on the top of gels 10 cm in length and run at 5 ma/gel for 5 to 6 hr, at which time the tracking dye had migrated to the bottom of the gel.

Processing of polyacrylamide gels. After electrophoresis, the gels were fixed in 20% (w/v) sulfosalicylic acid for 18 hr at room temperature and stained with 0.25% Coomassie blue for 3 hr. They were then washed and stored in 7% acetic acid.

Analysis of stained bands. Gels stained with Coomassie blue were scanned for absorption peaks at a wavelength of 610 or 620 nm with the aid of a model 2410 linear transport attachment to a model 240 spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio). Absorption curves were traced automatically with a Sargent model SRL linear-log recorder.

Analysis of radioactivity. After the stained gels were scanned, they were sliced transversely into segments of 1.25 mm each with the aid of a Chrambach gel slicer fabricated by Earl Sandbek Specialized Medical Instrumentation, Baltimore, Md. Each gel slice was then placed in a glass scintillation vial and depolymerized by adding 0.1 ml of 30% H_2O_2 and incubating overnight in a water bath at 50 C, essentially as described by Tishler and Epstein (22). This method has the great advantage of completely solubilizing the polymerized acrylamide and, at the same time, oxidizing the Coomassie blue dye. The solubilized acrylamide was then dissolved in 1 ml of NCS (Nuclear-Chicago Corp., Des Plaines, Ill.), diluted in toluene-based scintillation fluid, and counted in a Tri-Carb scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Quenching was consistently about 25%, and efficiency of counting was roughly 30% for ^{3}H and 60% for ^{14}C.

RESULTS

Polypeptides of Indiana serotype VS B virions grown in chick or mouse cells. Six stainable bands and six coincident peaks of radioactivity were seen after electrophoresis of purified ^{3}H- or ^{14}C-labeled VS_{Ind} B virions solubilized with acetic acid, 0.5 M urea, and SDS (Fig. 1). However, as many as 11 stainable bands were found when virion suspensions were incompletely purified. Moreover, variable patterns of band migration were frequently encountered in analysis of polypeptides released from impure virions that had been grown in CE, L, and primary rabbit kidney cells. At first, these results were thought to be attributable to incorporation of different cellular proteins into virions grown in cells of different species. The use of more refined techniques proved that these variable patterns of

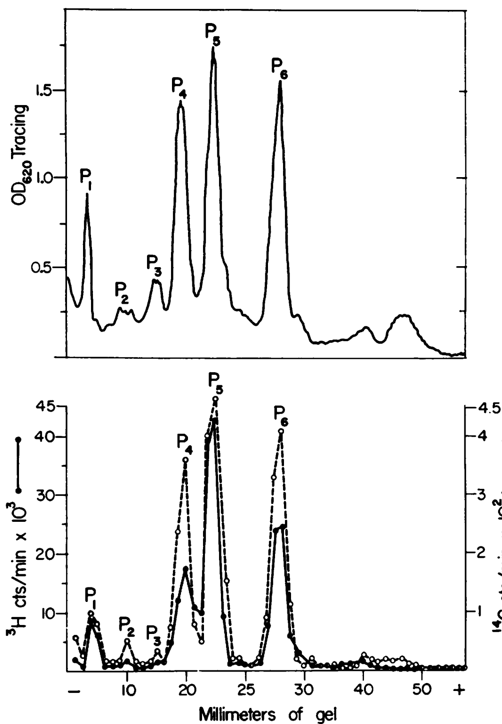

FIG. 1. *Electropherograms of polypeptides of VS_{Ind} purified B virions that had been grown in CE cells in the presence of ^{14}C-amino acids and in mouse L cells in the presence of ^3H-leucine plus ^3H-tyrosine. The proteins were extracted from a mixture of 100 µliters each of ^{14}C-virions (3,300 counts/min) and ^3H-virions (250,000 counts/min) with acetic acid, 0.5 M urea, SDS, and 2-mercaptoethanol. The gel contained 7.5% acrylamide, 0.5 M urea, and 0.1% SDS. Electrophoresis was continued for 5.5 hr at 5 ma. The upper tracing shows the OD scan at 620 nm after staining the protein bands with Coomassie blue. The same gel was then sliced into 1.25-mm lengths, each slice was depolymerized with 30% H_2O_2, dissolved in NCS, and suspended in toluene-based fluors; the ^3H and ^{14}C disintegrations were counted simultaneously. The window settings were such that no ^3H counts appeared in the ^{14}C channel and negligible ^{14}C counts appeared in the ^3H channel. The direction of migration is from left to right. Note the almost perfect coincidence of the six numbered peaks of OD, ^3H, and ^{14}C. The two unnumbered OD peaks that migrated most rapidly are artifacts at the gel front.*

polypeptide migration were due to inadequate purification. The contaminating proteins were shown to be of cellular origin by coelectrophoresis of extracts of purified virions with isotopically labeled proteins of uninfected cells.

As one additional test of the hypothesis that VS virions incorporate cellular proteins (3), separate pools of VS_{Ind} B virions were grown in CE cells in the presence of ^{14}C-amino acids and in L cells in the presence of ^3H-leucine and ^3H-tyrosine. Each pool of virions was concentrated and purified by differential and sucrose gradient centrifugation. Portions of 100 µliters each of CE virions and L virions were mixed, and the polypeptides were extracted and subjected to coelectrophoresis on a single gel. Figure 1 shows representative patterns of stained bands and each radioactive label in the same gel.

It is apparent that 0.5 M urea, 1% SDS, and mercaptoethanol dissociated six species of polypeptides from B virions, which migrated identically regardless of whether the virions were grown in CE or L cells. Peaks 4, 5, and 6 represent major polypeptide components, whereas peaks 1, 2, and 3 are minor components.

This experiment suggests that none of the six polypeptides is of cellular origin. If cellular proteins were incorporated into VS virions, they are identical for both chick and mouse cells. It is possible, of course, that cellular proteins cannot be detected by this method, but that they could be detected by more sensitive immunological techniques (3). Several attempts were also made to demonstrate incorporation of cellular proteins into virions by growing the virus in cells prelabeled with ^3H- or ^{14}C-amino acids and chasing with excess cold amino acids at the time of infection. The results of the experiments were inconclusive because of a persistent pool of labeled amino acids, but it was clear that none of the six identifiable polypeptides is of cellular origin.

Molecular weight estimates. Electropherograms of proteins analyzed by the method of Maizel et al. (14, 15) permit estimations of relative molecular weights by virtue of the fact that SDS minimizes the native charge differences of the polypeptides. To this end, marker proteins and the polypeptides extracted from VS_{Ind} B virions were analyzed by the techniques used by Shapiro et al. (18) of electrophoresis in 5% polyacrylamide containing 0.1% SDS. Accurate representation of relative migration could be made by the refined technique of scanning the protein peaks in gels stained with Coomassie blue at a wavelength of 610 nm. The most suitable marker proteins of known molecular weight and purity turned out to be cytochrome *c*, trypsin, ovalbumin (all from Sigma Chemical Co., St. Louis, Mo.), pepsin (Mann Research Laboratories, New York, N.Y.), and bovine serum albumin (BSA) (Calbiochem, Los Angeles, Calif.).

Figure 2 shows the linear relationship of the logarithm of molecular weight of each marker protein plotted against the relative distance of migration using cytochrome *c* as the standard.

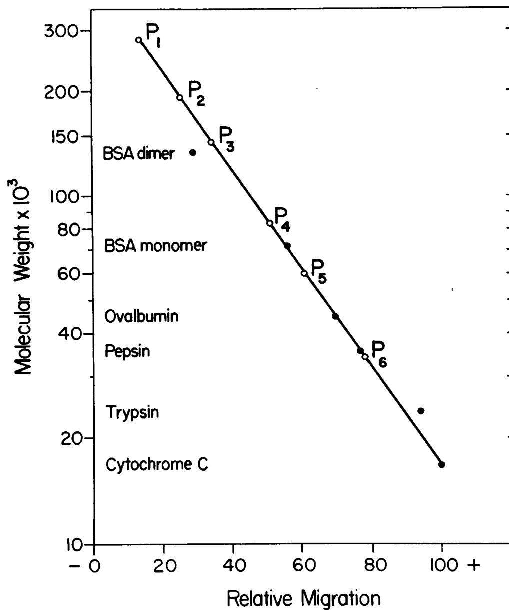

FIG. 2. *Semilog plot of molecular weight against distance of migration after electrophoresis (5 ma/gel, 5.5 hr) of marker proteins and VS_{Ind} proteins in 5% polyacrylamide gels in phosphate buffer, pH 7.2, and 0.1% SDS. The gels were stained with Coomassie blue and the position of OD_{610} peaks determined with the gel scanner and automatic recorder. Relative migration was measured as the distance from the origin (cathode) of each peak with cytochrome c as an arbitrary standard of 100. All marker proteins gave single sharp peaks, with the exception of BSA which showed distinct peaks for the monomer and dimer. Relative migration of ^3H-VS_{Ind} protein peaks (P1–P6) was determined by both optical density and radioactivity after electrophoresis of virion proteins solubilized with acetic acid, 0.5 M urea, and SDS.*

Similar values were obtained when the marker proteins were run individually or together. The relative migration of VS virion polypeptides analyzed by electrophoresis concurrently in a 5% gel under identical conditions is also illustrated in Fig. 2. Molecular weights of the virion polypeptides (P1–P6) were calculated from the distance of migration of each peak and the slope of the linear portion of the curve, assuming that the relative mobilities are inversely proportional to the logarithm of the molecular weights. The following molecular weight values were obtained for the six VS_{Ind} B virion proteins: P6 ≅ 34,500, P5 ≅ 59,500, P4 ≅ 81,500, P3 ≅ 140,000, P2 ≅ 186,000, and P1 ≅ 275,000. Since the reliability of the procedure is greatest for proteins of molecular weights in the range 15,000 to 90,000 (18), the estimates for the minor virion polypeptides P1, P2, and P3 can be considered only approximate.

Dissolution of virions in 8 M urea. There is little precedent for the finding of single polypeptide chains of such large molecular weight as those of the minor proteins of VS virions. The presence of mercaptoethanol in the solvent made it seem unlikely that the proteins of high molecular weight were polypeptide chains linked by disulfide bonds. We assumed, therefore, that P1, P2, and P3 were aggregates or polymers of P4, P5, and P6 that were incompletely dissociated in 0.5 M urea. Therefore, purified virions were solubilized in acetic acid, 8 M urea, and 1% SDS and dialyzed overnight against phosphate buffer containing 8 M urea, 0.1% SDS, and 0.1% 2-mercaptoethanol. When this protein mixture was electrophoresed on a 7.5% polyacrylamide gel containing 8 M urea as well as SDS and mercaptoethanol, the individual polypeptides were incompletely resolved, presumably because of alteration in the sieving properties of the gel. Nevertheless, P1 seemed to be present, albeit in reduced amount.

When purified virions dissolved in 8 M urea were run on a 5% polyacrylamide gel containing 0.5 M urea, P1 persisted in an amount equivalent to that of P1 dissociated from VS virions by 0.5 M urea, but P2 and P3 were missing or reduced in amount (Fig. 3). From these data, we tentatively conclude that P2 and P3 are polymers or mixed aggregates of the smaller polypeptides, but that P1 may represent a very large single polypeptide chain or, conceivably, a number of disulfide-bonded chains.

Also noted in Fig. 3 is the suggestion that P4 may be two incompletely resolved polypeptides. This 5% polyacrylamide gel may have greater resolving power than 7.5% gels.

Comparison of the B virion proteins of the Indiana and New Jersey serotypes. The antigenic unrelatedness of VS_{Ind} and VS_{NJ} viruses (16; R. R. Wagner, *unpublished observations*) suggested that distinct structural polypeptides might be present in each type of virion. Accordingly, electrophoretic patterns were examined after urea-SDS dissolution of polypeptides prepared from purified VS_{NJ} virions. Six distinct protein peaks were separated that migrated in a fashion indistinguishable from those of fractionated VS_{Ind} virions. To detect subtle differences in protein profiles, each serotype virus was labeled with a different isotope and analyzed on the same electropherograms.

VS_{Ind} virions were grown in CE cells in the presence of ^3H-leucine and ^3H-tyrosine, and VS_{NJ} virions were grown in the presence of ^{14}C-amino acids. B virions from each preparation were

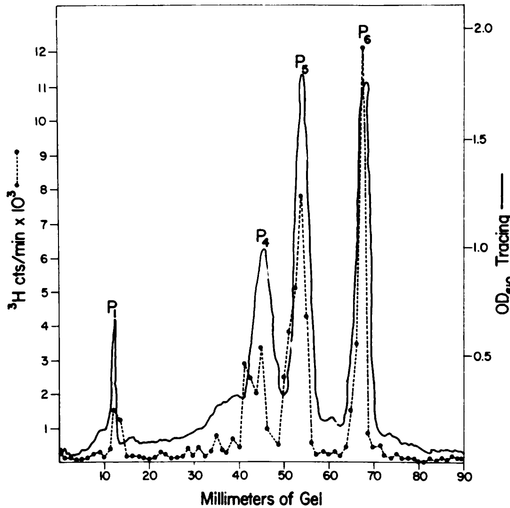

FIG. 3. *Electropherogram of ^3H-VS_{Ind} B virion proteins dissociated in 8 M urea as well as acetic acid, SDS, and 2-mercaptoethanol, and electrophoresed for 5.5 hr at 5 ma on a 5% polyacrylamide gel containing 0.5 M urea and 0.1% SDS. Scans for stainable bands and radioactive analysis were made as in Fig. 1. Note the more rapid migration of P1, P4, P5, and P6 as compared with a 7.5% gel and the apparent absence or reduction in P2 and P3. The trailing optical density shoulder and split radioactive peak in the region of protein number 4 may be evidence of an additional poorly resolved protein.*

concentrated and purified on sucrose gradients, and the polypeptides were solubilized. A 100-µliter amount of each preparation was mixed and subjected to coelectrophoresis. The gels were stained, scanned at OD_{620}, and sectioned for simultaneous counting of ^{3}H and ^{14}C disintegrations.

Figure 4 reveals six identifiable peaks of optical density and six corresponding peaks of radioactivity. Only polypeptide 6 of the two serotypes showed different electrophoretic mobility; P6 of VS_{NJ} ran two fractions (2.5 mm) ahead of P6 of VS_{Ind}.

Identity of structural proteins of B and T VS_{Ind} virions. Previous studies had shown that the major antigenic component(s) of separated infectious B and defective T particles of VS_{Ind} virus could not be distinguished by cross-complement-fixation and cross-neutralization tests (8). Nevertheless, it seemed possible that the defectiveness of T particles could be attributable to absence or alteration of one or more structural polypeptides. Therefore, an analysis was made of the electrophoretic profiles of B and T polypeptides labeled with different isotopes and run on the same gel.

VS_{Ind} B virions were grown in CE cells infected at a multiplicity of 5 and labeled with ^{14}C-amino acids. T virions were produced by infecting CE cells at a multiplicity of 50 in the presence of ^{3}H-leucine and ^{3}H-tyrosine. The ^{14}C-B and ^{3}H-T virions were purified separately by two cycles of rate zonal sedimentation in linear 0 to 40% sucrose gradients. These purified virions were then mixed and subjected to equilibrium sedimentation in CsCl at a starting density of 1.23 g/ml. Figure 5 shows the coincident peaks of ^{3}H and ^{14}C radioactivity at a CsCl density of 1.20 mg/ml. This experiment corrects our previous report of slight differences in the buoyant density of B and T particles (8).

Fraction 10, collected from the CsCl gradient (Fig. 5) which contained both ^{3}H-T and ^{14}C-B virions, was treated with acetic acid, urea, and SDS. The resultant polypeptides were analyzed by gel electrophoresis. Figure 6 demonstrates unequivocally that B and T virions share six

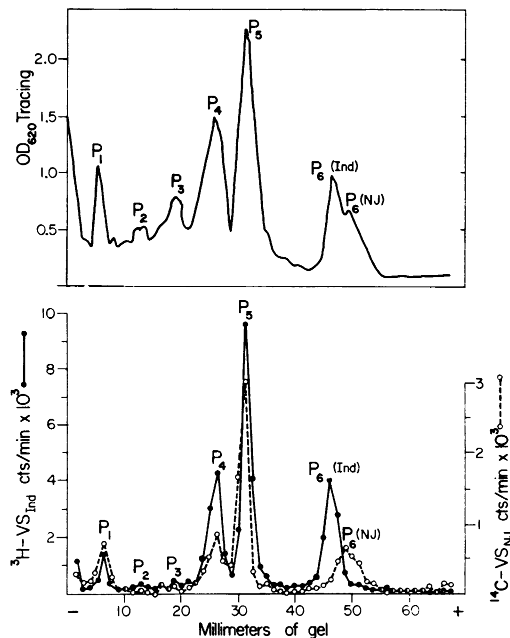

FIG. 4. *Comparative electrophoretic migration of the proteins of purified B virions of VS_{Ind} virus grown in CE cells in the presence of ^3H-leucine and ^3H-tyrosine and of VS_{NJ} B virions grown in CE cells in the presence of ^{14}C-amino acids. ^3H-VS_{Ind} virions (100 µliters, 49,000 counts/min) were mixed with ^{14}C-VS_{NJ} virions (100 µliters, 13,000 counts/min), dissociated in acetic acid, 0.5 M urea, SDS, and 2-mercaptoethanol, and analyzed by coelectrophoresis on a 7.5% polyacrylamide gel. The stained gels were analyzed by scanning at OD_{620} and by counting of both isotopes in slices of 1.25 mm each.*

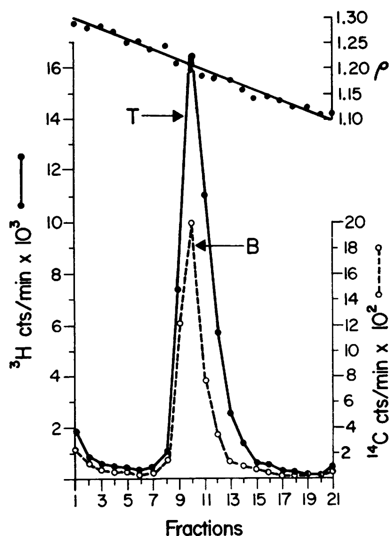

FIG. 5. *Cocentrifugation in CsCl of VS_{Ind} T virions labeled with ^{3}H-leucine and ^{3}H-tyrosine and B virions labeled with a uniform mixture of ^{14}C-amino acids. Both ^{3}H-T and ^{14}C-B virions were separately purified by differential and rate zonal centrifugation; 100 μliters of each was mixed with CsCl (starting specific gravity 1.23 g/ml) and centrifuged to equilibrium for 72 hr at 38,000 rev/min in an SW50 rotor. Fractions of 0.2 ml each were collected from the bottom of the tube and 50-μliter portions were weighed and assayed for radioactivity.*

polypeptides that are indistinguishable by this method of analysis.

Preparative gel electrophoresis. Several attempts were made to purify the individual proteins of VS virions by preparative gel electrophoresis. For this purpose we used the Buchler "Fractophorator" (Buchler Instrument Co., Fort Lee, N.J.) with a 7.5% (or 5%) polyacrylamide gel (18 mm in diameter, 20 mm in length) prepared in the same way as that for analytical gel electrophoresis. This apparatus is designed for elution of proteins at the gel front into a lower buffer reservoir. Polypeptides extracted from purified VS_{Ind} B virions, ~2 mg of protein in 0.4 ml containing 20 μliters of 1% bromophenol blue, were layered on the gel and electrophoresed at 40 ma (constant current) for 16 hr. The lower reservoir contained 0.1 M phosphate buffer (pH 7.2) and the upper reservoir contained the same buffer with 0.1% SDS. After the tracking dye reached the bottom of the gel, fractions of 2 ml each were collected every 10 min with the aid of a time-operated Buchler fraction collector. Radioactivity was counted in 50-μliter portions of each fraction.

Results obtained by preparative gel electrophoresis of polypeptides extracted from VS_{Ind} B virions are shown in Fig. 7A. Clean peaks of radioactivity representing individual polypeptides were not obtained as readily under the conditions of this procedure as they were by analytical gel electrophoresis. Nevertheless, peak fractions of ^{14}C radioactivity were concentrated to 1 ml by pressure dialysis and rerun on an analytical gel along with marker ^{3}H-labeled polypeptides extracted from whole VS_{Ind} virions. No identifiable polypeptides were found in pooled fractions 11 and 12 collected from the preparative gel electropherogram (Fig. 7A). However, fraction 26 contained only polypeptide 6 (Fig. 7B) and fractions 41–42 and 44 contained only polypeptide 5 (Fig. 7C). Preparative gel fractions 58 and 62 each contained polypeptides 4 and 5. No ^{14}C radioactivity representing polypeptides 1, 2, or 3 was eluted from the preparative gel. Preparative gels stained with Coomassie blue after electrophoresis for 16 hr still showed two or three protein bands.

It appears, therefore, that polypeptides 6 and 5, which are relatively low in molecular weight, can be purified to some extent by preparative gel electrophoresis. However, other gel and buffer conditions are required for clean separation of proteins of VS virions in sufficient quantity for analysis of tryptic peptides and amino acids.

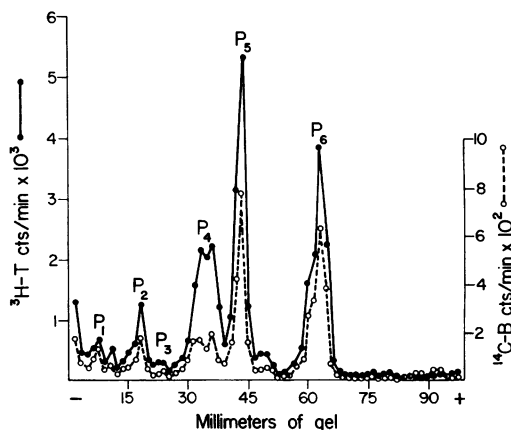

FIG. 6. *Coelectrophoresis on a 7.5% polyacrylamide gel of the proteins of VS_{Ind} T virions labeled with ^{3}H-leucine and ^{3}H-tyrosine (44,700 counts/min) and VS_{Ind} B virions labeled with ^{14}C-amino acids (5,900 counts/min). The mixture of purified virions was collected from fraction 10 of the CsCl gradient shown in Fig. 5, and the proteins were extracted as described in the legend to Fig. 1.*

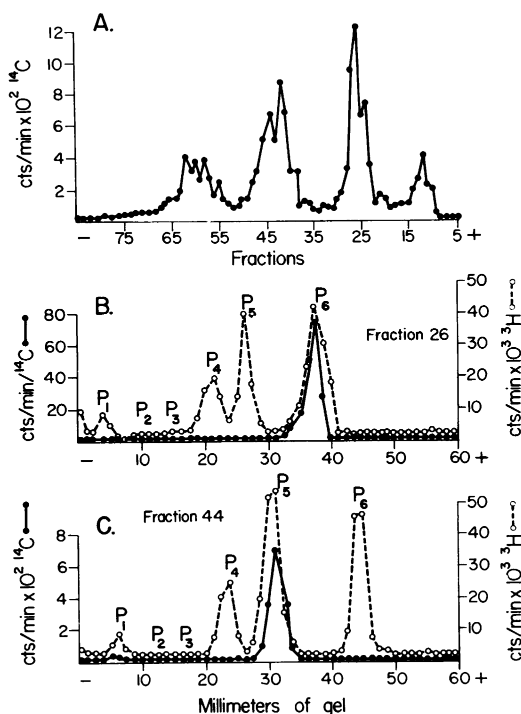

FIG. 7. *Preparative gel electrophoresis of purified VS_{Ind} B virions labeled with ^{14}C-amino acids. (A) Electrophoretic profile of labeled proteins dissociated in acetic acid, 0.5 M urea, and SDS, and eluted from the preparative gel front. Fractions of 2 ml each were collected every 10 min from a 7.5% polyacrylamide gel, 20 cm in length. The tracking dye was eluted in fraction 1. (B) Coelectrophoresis on a 7.5% analytical gel of fraction 26 from the preparative gel shown in A along with marker ^{3}H-proteins extracted from VS_{Ind} virions. (C) Coelectrophoresis on a 7.5% analytical gel of fraction 44 from the preparative gel shown in A along with marker ^{3}H-VS_{Ind} proteins.*

DISCUSSION

Holland (4) reported that electrophoretically identical viral polypeptides are synthesized in cells of different animal species infected with mengovirus and analyzed on polyacrylamide gels in the presence of SDS and urea. The same type of correspondence was found in our studies of structural polypeptides of VS_{Ind} virions grown in chick and mouse cells. Our original expectation was that enveloped VS virions, which mature at the cell surface and appear by electron microscopy to incorporate host cytoplasmic membrane in their envelopes, would differ from picornaviruses in this respect. Moreover, the immunological evidence presented by Cartwright and Pearce (3) suggested the presence of specific host cell components acquired by VS virions grown in hamster and pig cells and purified by sucrose gradient centrifugation. Our finding of identical electrophoretic profiles of dissociated VS virions that had been grown in chick or mouse cells indicates that none of the six identifiable VS virion proteins is of cellular origin. It is possible, of course, that chick and mouse cells share a common cytoplasmic membrane protein or that minor cellular components cannot be detected by gel electrophoresis but might be revealed by more sensitive immunological methods. It is also difficult to interpret the significance of our failure to demonstrate prelabeled cellular proteins chased into VS virions. This finding could merely be a manifestation of rapid turnover of cellular membrane proteins rather than their replacement by viral proteins. Alternatively, perhaps there are no cellular proteins incorporated in the VS virions.

The virtually identical electrophoretic profiles of the structural proteins dissociated from infectious B and defective T virions of VS virus support the hypothesis that T is merely a truncated form of B (8). Presumably, the length of the T virion (~65 nm compared with ~180 nm for the length of B) is determined by the length of its RNA, which is approximately one-third the length of the RNA of B (7). Recent evidence suggests that T virions contain a helical nucleoprotein which is also one-third the length of the nucleoprotein of B (17). Hence, one might assume that all the other structural components of the T virion are proportionately the same as those of B but scaled down to one-third the amount. It is not surprising, therefore, that B and T virions have the same buoyant density and the same relative proportions of each structural polypeptide.

Despite major antigenic differences, the structural polypeptides of adenovirus types 2, 7a, and 12 reveal only minor differences in electrophoretic profiles in SDS gels (15), as do the structural polypeptides of reovirus types 1, 2, and 3 (10). Similar patterns were obtained with the two antigenically distinct VS viruses. Five of the six polypeptides of the New Jersey serotype migrated in urea-SDS gels identically to the corresponding five proteins (P1–P5) of Indiana virions. The slightly more rapid migration of New Jersey protein 6 may represent a considerable difference in amino acid content and, hence, a conformational change that could account for marked antigenic dissimilarity to Indiana protein 6. It seems likely that protein 6 is the surface antigen which accounts for the complete lack of cross-neutralization of VS_{Ind} and VS_{NJ} virus by their respective antisera (Wagner, *unpublished*

data). Additional evidence that P6 is the surface protein will be presented in a subsequent communication.

Compared with other viruses, the VS virion appears to be intermediate in the complexity of its protein composition. The vaccinia virion contains at least 17 structural polypeptides (6), the adenovirion 9 (15), and the reovirion 7 (10). Among the picornaviruses, poliovirions have four polypeptides (14) and others may have fewer (4, 5). Of considerable interest is the reported finding that the Sindbis arbovirion seems to contain only two structural polypeptides, one associated with an easily removed lipid-containing envelope and another which remains with the core (20), which would make this virus as simple in its protein structure as small RNA phages (23).

The six proteins identified in fractionated VS_{Ind} virions have a combined molecular weight of almost 800,000 daltons. This figure turns out to be far too large to satisfy the requirement that a separate cistron of the VS virus genome codes for each of six polypeptides, even if one overlooks the unlikelihood of single polypeptide chains of such enormous length. The molecular weight of the single-stranded RNA molecule isolated from the infectious B virions of VS virus has been estimated to be no greater than 4×10^6 daltons (7, 17), which should provide genetic information to code for individual polypeptides that total no greater than 400,000 daltons, assuming a coding ratio of 900 daltons for each codon to 90 daltons for one amino acid of average size (14). In addition, this estimate makes no provision for synthesis of nonstructural proteins. By comparison, the four structural polypeptides of poliovirions have a combined molecular weight of 93,000 daltons; the poliovirus genome (2×10^6 daltons) could code for polypeptides totaling 200,000 daltons (14). However, a total of 14 capsid and noncapsid poliovirus proteins with an aggregate molecular weight of about 500,000 daltons has been identified in infected cells. Recent data reported by Summers and Maizel (21), Jacobson and Baltimore (9), and Holland and Klein (5) indicate that poliovirus proteins may be synthesized as high-molecular-weight components that are cleaved into smaller polypeptides before incorporation into virion capsids. A similar situation may obtain for VS virions, with the exception that a relatively small proportion of uncleaved protein may be incorporated into virions. Such a large polypeptide could play a special role in the structural organization of these complex virions.

It seems likely that the minor polypeptides P2 and P3 of VS virions are aggregates or polymers of polypeptides P4, P5, and P6 whose combined molecular weight is approximately 175,000 daltons. This figure for the three major polypeptides represents a reasonable estimate for coding of structural polypeptides by the VS virus genome. P2 could be an aggregate of P4, P5, and P6 or a polymer of any of these individual polypeptides. Similarly, P3 (molecular weight $\cong$ 145,000) could be an aggregate of P5 (molecular weight $\cong$ 59,500) and P4 (molecular weight $\cong$ 81,500) or a polymer of any of the major polypeptides. P1 is more difficult to explain because it is larger than the combined molecular weight of P4, P5, and P6; also, P1 does not dissociate to any appreciable extent in 8 M urea, suggesting that it is not a simple aggregate. Moreover, P1 is remarkably consistent in its electrophoretic behavior on acrylamide gels, and, conceivably, could be an uncleaved covalently bonded product of the polycistronic message of VS virus that is incorporated into the virion.

Studies now in progress are designed to identify the intracellular prescursor proteins and to localize the three major and the three minor polypeptides by controlled degradation of VS virions.

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