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Polypeptide Composition of Extracellular Enveloped Vaccinia Virus

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Extracellular enveloped vaccinia (EEV) virus grown in SIRC and in HeLa cells was purified by consecutive equilibrium centrifugations in sucrose and cesium chloride gradients. A higher degree of purity was obtained with virus material prepared in SIRC cells. The polypeptides of purified EEV and INV (intracellular naked vaccinia) virus were compared in polyacrylamide slab gel electrophoresis. Three proteins (200,000 molecular weight [200K], 95K, and 13K) detected in HeLa-derived INV were absent in EEV. In addition, two INV proteins (65K and 30K) occurred in reduced concentrations in EEV, while another INV protein (27K) was increased in EEV. INV from SIRC cells showed similar alterations of these proteins (with the exception of the 30K and 13K proteins). Detergent treatment, ether extraction, and Pronase treatment showed that these six proteins are located at the surface of INV and are not necessary for infectivity. Eight proteins (210K, 110K, 89K, 42K, 37K, 21.5K, 21K, and 20K) were detected in EEV that were absent from INV. Brij-58 treatment was employed to remove the envelope from EEV, resulting in the formation of naked particles and an envelope fraction which were separated on cesium chloride gradients. The envelope fraction contained all eight proteins. Seven of the eight proteins were glycoproteins, with the 37K protein being the only unglycosylated protein. It is concluded that a processing of surface INV particle proteins occurs during envelopment. The resultant EEV particle is comprised of an INV particle with a modified surface composition enclosed in an envelope containing virus-specific proteins unique to EEV.

Vaccinia virus has a complex structure which has been analyzed by polypeptide separation in polyacrylamide gel electrophoresis (PAGE). Discontinuous buffer systems originally revealed at least 30 structural polypeptides (13, 17). More recently, in excess of 40 polypeptides have been discerned (11, 19, 20). The location of several of these polypeptides has been established (17, 19). These studies have dealt exclusively with vaccinia virus purified from cytoplasmic extracts of infected cells which we have termed intracellular naked vaccinia (INV) virus particles (14). Although tissue culture-propagated vaccinia is largely a cell-associated virus, a small proportion of mature virus particles acquire an envelope either at intracellular membranes or at the cytoplasmic membrane (8). The envelope of extracellular enveloped vaccinia (EEV) virus particles has been shown to contain antigenic component(s) distinct from INV antigens (1, 4, 16, 22) and to contain the vaccinia hemagglutinin (14). In addition, the presence of an envelope has been shown to result in the adsorption and penetration of EEV in a manner distinct from that of INV (15).

The antigenic and biological characteristics that distinguish EEV from INV, coupled with the fact that the dissemination of infection in vitro and presumably in vivo (3) is mediated by EEV, have prompted us to continue our studies of this extracellular virus product. In this paper we report results from studies of the polypeptide composition of EEV analyzed by PAGE.

MATERIALS AND METHODS

Virus growth and purification. The IHD-J strain of vaccinia (obtained from The Public Health Research Institute, New York, N.Y.) was produced in large quantities by infection of stationary cultures of SIRC cells and HeLa cells at a multiplicity of infection of 0.1 PFU/cell. Cells were infected at room temperature with 1 ml of virus suspension per Roux bottle (ca. 8×10^7 cells) for 30 min, after which the unadsorbed virus was removed and 100 ml of Eagle minimal essential medium plus 2% calf serum was added per Roux bottle. Virus proteins were radioactively labeled by the inclusion of 3 μCi of [^{3}H]leucine per ml. [^{3}H]Fucose or [^{3}H]glucosamine (3 μCi/ml; New England Nuclear) was used to label glycoproteins. At 48 h postinfection, the cells and large debris were removed by a 5-min centrifugation at 5,000 rpm in a Sorvall

GSA angle rotor (Du Pont Instruments). EEV was sedimented by centrifugation of the medium at 10,000 rpm for 15 min in the GSA angle rotor. The EEV-containing sediment was suspended in phosphate-buffered saline. To obtain INV, the cells were suspended in distilled water (1 ml per Roux bottle) for 10 min and then homogenized with 10 strokes in a Dounce homogenizer. The large debris and nuclei were sedimented by centrifugation at 3,000 rpm for 10 min. Both INV and EEV were treated with a Rapidis (Ultrasonic Ltd.) Sonifier at 30 μM for 10 s before purification.

EEV was partially purified by equilibrium centrifugation for 60 min at 30,000 rpm on 9-ml linear 30 to 60% (wt/wt) continuous sucrose gradients in an SW40 rotor. Bands were collected through the bottom of the tube and diluted 1:4 with phosphate-buffered saline. EEV was subsequently purified by centrifugation in cesium chloride for 1 h at 25,000 rpm in an SW27 rotor. The gradients were formed by prelayering 1.30- (6 ml), 1.25- (8 ml), and 1.20-g/ml (10 ml) cesium chloride solutions.

INV was purified by rate zonal sedimentation for 30 min at 15,000 rpm on 32-ml linear 20 to 45% (wt/wt) continuous sucrose gradients in an SW27 rotor. The band of INV particles was harvested, diluted, and centrifuged in cesium chloride as described for EEV. The INV and EEV virus, banding at 1.27 g/ml and 1.23 g/ml, respectively, were collected and frozen at −70°C (14).

Virus quantitation. Virus was plaqued on monolayers of A-549 cells as previously described (14). The protein content of purified virus suspensions was determined by UV spectrophotometry. One optical density unit at 260 nm is equal to 64 μg of protein (9).

Isolation of the envelope from EEV. Purified EEV (1 ml; 60 or 100 μg) in phosphate-buffered saline was exposed to Brij-58 detergent at a final concentration of 1% for 10 min at room temperature with intermittent agitation. The released envelope material and virus particles were separated on a gradient formed by prelayering 1.30- (3 ml), 1.25- (4 ml), and 1.20-g/ml (5 ml) cesium chloride solutions. Centrifugation was for 30 min at 30,000 rpm in an SW40 rotor. The released material was removed from the top of the gradient by a Pasteur pipette, and the banded virus particles were collected dropwise after puncturing the bottom of the centrifuge tube.

Treatment of INV with detergents, ether, and Pronase. Samples of purified INV (1 ml; 60 or 100 μg) in phosphate-buffered saline were sonically disrupted and subjected to the following treatments. Particles were exposed to either (i) 1% Brij-58 detergent under conditions described above for EEV; (ii) 1% Cutscum detergent at room temperature for 30 min; (iii) 0.5% Nonidet P-40 (NP-40) at 37°C for 60 min (7, 17); (iv) 0.12% Tween 80 for 5 min in an ice bath, then extracted with an equal volume of cold ether for 15 min (12); or (v) 10 μg of Pronase per ml for 60 min at 37°C. Following all treatments, virus particles were separated from the released material by centrifugation in discontinuous cesium chloride gradients as described above for Brij-58-treated EEV.

Preparation of material for PAGE. Virus particles (60 or 100 μg) were precipitated with 10% trichloroacetic acid. Solubilized material was trichloroacetic acid precipitated in the presence of 200 μg of insulin per ml. Insulin was included as a carrier protein since it migrates at the front in PAGE and thus does not disturb the virus protein pattern. Precipitated material was centrifuged in an angle rotor at 7,000 rpm for 30 min at 4°C. The supernatant was removed, and the tubes were recentrifuged for 5 min to clear the protein film of any remaining trichloroacetic acid. The protein film was then washed with −20°C ether for dehydration. The material was suspended in a dissociating buffer consisting of 0.0625 M Tris-H_3PO_4 (pH 6.8), 2% sodium dodecyl sulfate, 0.2% dithiothreitol, 0.1% EDTA-Na_2, and 10% glycerol and immersed in boiling water for 3 min to complete dissociation.

PAGE. The dissociated proteins were separated on a slab gel apparatus (21). The separation gel consisted of 15% acrylamide and 0.18% *N,N'*-methylene-bisacrylamide. The spacer gel was 4.5% acrylamide, 0.12% *N,N'*-methylene-bisacrylamide, and 0.6% agarose. The buffer system was as previously reported (10) except for the substitution of Tris-H_3PO_4 for Tris-hydrochloride in the spacer gel. The gels were polymerized by addition of *N,N,N',N*-tetramethylenediamine and ammonium persulfate to give a final concentration of 0.03% of each in the separation gel and 0.06% of each in the spacer gel. The 60-μg samples of virus protein were analyzed in 3-mm channels, and 100-μg samples were analyzed in 5-mm channels. Electrophoresis was performed at room temperature in gels (8 by 12 by 0.15 cm) for 4 to 5 h at 3 to 4 W. Gels were stained overnight in 0.25% Coomassie brilliant blue in 35% ethanol-10% acetic acid in water and destained in the same solvent lacking the stain. Gels for scintillation autofluorography were impregnated with 2,5-diphenyloxazole before drying and exposure to X-ray film (2).

Materials. The following detergents were used: Brij-58 (Atlas Chemical Co.), NP-40 (Shell Chemical Co.), and Cutscum (Fisher Scientific Co.). The Pronase (Calbiochem) had a specific activity of 86,000 proteolytic units $Kunit_3$ per g. The reference proteins used were myosin (220,000 molecular weight), β-galactosidase (135,000), phosphorylase (94,000), bovine serum albumin (67,000), actin (44,000), chymotrypsinogen (25,000), and cytochrome *c* (12,400).

RESULTS

Purification of EEV. Several purification schemes were compared as to EEV yield and purity. Centrifugation of EEV on a continuous equilibrium sucrose gradient (30 to 60% [wt/wt] in an SW40 rotor) at 30,000 rpm for 60 min or on a continuous sedimentation sucrose gradient (20 to 45% [wt/wt] in an SW27 rotor) at 15,000 rpm for 45 min, both followed by an equilibrium cesium chloride gradient, resulted in EEV preparations that did not differ in their PAGE protein patterns. However, the purification by equilibrium sucrose centrifugation yielded two to five times as much EEV as the sucrose sedimentation. The latter method resulted in most of the virus being lost from the EEV band and appearing in the pellet. Centrifugation of EEV in an

SW40 rotor at 30,000 rpm for 60 min on discontinuous (30 to 60% [wt/wt]) sucrose gradients yielded as much EEV as was recovered from the continuous (30 to 60% [wt/wt]) sucrose gradient, but it was of inferior purity (Fig. 1). EEV from discontinuous sucrose gradients contained one protein band absent from continuous sucrose gradient-purified EEV. This band comigrated with the cellular protein actin. This protein varied in quantity from preparation to preparation, whereas the other proteins were invariable, and therefore this protein served as an indicator of cellular contamination of the EEV preparations.

Protein analysis of EEV and INV from HeLa and SIRC cells. To determine the protein composition of EEV, we compared purified EEV and INV from vaccinia virus strain IHD-J. Viruses from the SIRC fibroblast cell line and the HeLa epithelial cell line were investigated (Fig. 2). More than 30 INV proteins were readily discernible. Both the quantity and distribution in the gel of INV proteins from HeLa cells closely agree with the most recent published results (19, 20). INV proteins in the 58,000-molecular-weight (58K) to 65K range could be resolved visually, but this was often not possible in photographs of gels. A similar INV protein pattern was found for vaccinia strains WR and Lister grown in HeLa cells (gel not shown).

An interesting difference was observed between INV preparations from HeLa and SIRC cells. Proteins 200K, 95K, and 65K were very much reduced in INV from SIRC. The 27K SIRC INV protein concentration was by contrast significantly increased. The 30K and 13K proteins appeared in the same concentration. This resulted in a SIRC cell INV protein composition that was more similar to EEV from both HeLa and SIRC than it was to INV from HeLa (see below). These molecular differences may be related to the finding that, although HeLa and SIRC release approximately equal quantities of EEV, the number of INV particles that remain cell associated in HeLa cells is five times that found in SIRC cells. This quantitative difference is also the basis for the use in the following experiments of INV purified from HeLa cells.

The EEV proteins from both SIRC and HeLa cells differed markedly from the HeLa cell INV protein pattern. The INV proteins at 200K, 95K, and 13K were absent from EEV. In addition, proteins 65K and 30K were significantly reduced in EEV compared to INV, whereas protein 27K was increased. EEV proteins from HeLa and SIRC also differed from each other. Several proteins were consistently found in HeLa cell EEV that were absent from SIRC cell EEV. The heaviest-staining protein comigrated with actin,

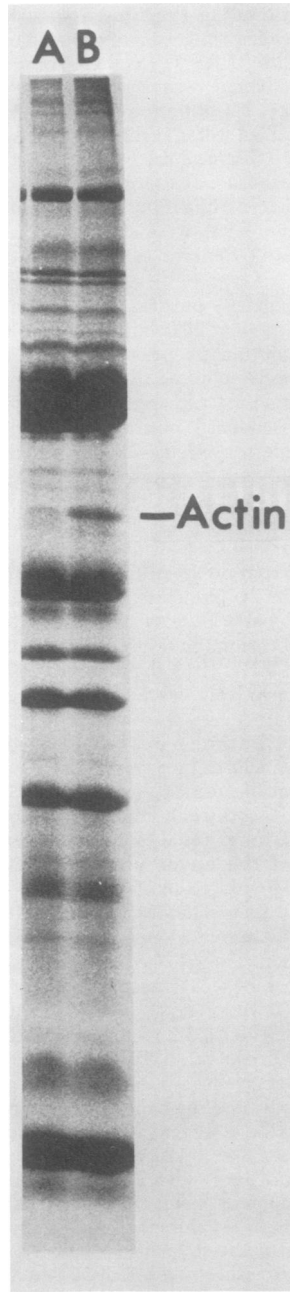

FIG. 1. *PAGE analysis of EEV from SIRC cells purified by centrifugation on a continuous 30–60% (wt/wt) sucrose gradient (A) or a discontinuous 30–60% (wt/wt) sucrose gradient (B) followed by an equilibrium cesium chloride gradient centrifugation as described in the text.*

and presumably these extra proteins represent contaminating HeLa cell protein. We were unable to further purify HeLa cell EEV using a variety of centrifugation techniques. As a result,

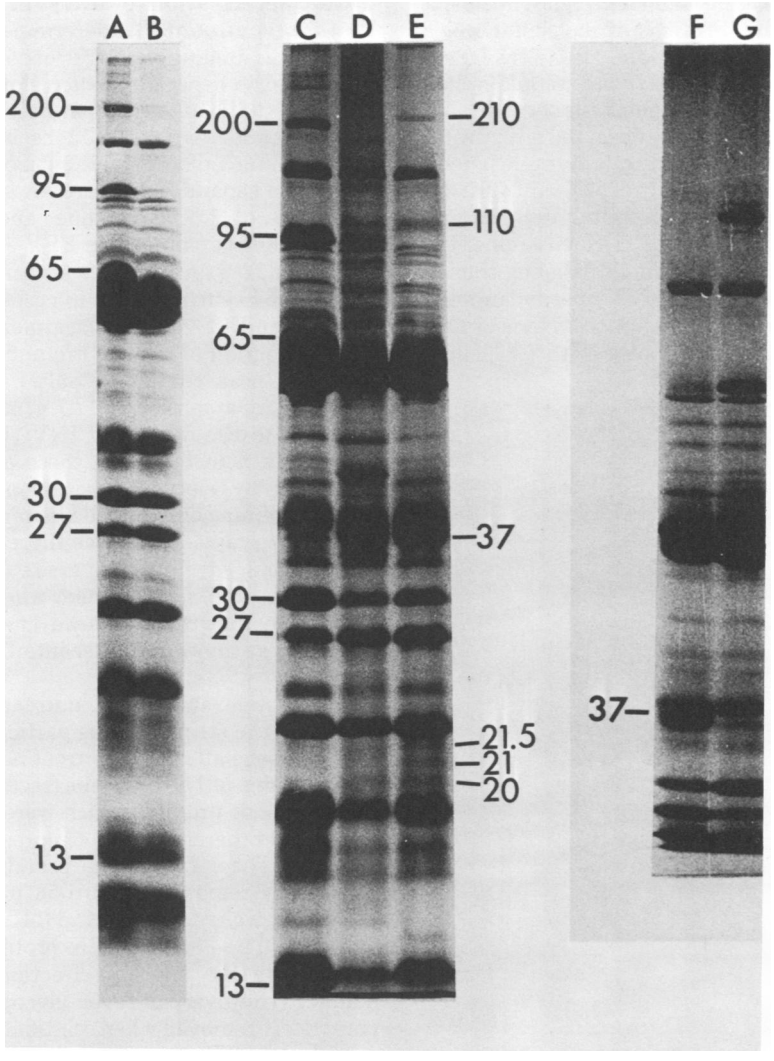

FIG. 2. *A 15% (A-E) and a 10% (F, G) PAGE analysis of INV and EEV. INV from HeLa (A, C, G) was compared with INV from SIRC (B), EEV from HeLa (D), and EEV from SIRC (E, F).*

the EEV employed in the following experiments was mostly derived from SIRC cells.

In addition to the quantitative changes seen in certain INV proteins in EEV particles, Fig. 2 shows that EEV particles contain some proteins not demonstrable in INV. These additional proteins were most readily discerned in EEV purified from SIRC cells, whereas the level of cellular protein contamination, particularly in the high-molecular-weight range, of HeLa cell EEV made the detection of these proteins difficult. Six EEV-specific proteins were detected by Coomassie brilliant blue staining. Five had molecular weights of 210K, 110K, 21.5K, 21K, and 20K and did not comigrate with any INV protein. These proteins are also clearly visible in Fig. 1. A sixth protein at 37K comigrated in 15% PAGE with an INV protein. However, they could be differentiated in 10% PAGE (Fig. 2E and F).

Glycoproteins of EEV. The glycoprotein composition of EEV was analyzed by PAGE of [^{3}H]fucose-labeled virus. We used [^{3}H]fucose instead of [^{3}H]glucosamine for two reasons. First, preliminary experiments showed that two INV proteins were labeled by [^{3}H]glucosamine but not by [^{3}H]fucose, in agreement with previous work (5, 6, 17). This permits the specific labeling of only EEV proteins by [^{3}H]fucose. Second, the 110K EEV protein could not be labeled by [^{3}H]glucosamine, whereas [^{3}H]fucose was incorporated. Figure 3 shows a scintillation autofluorogram of [^{3}H]fucose-labeled EEV from both

HeLa and SIRC cells and HeLa cell INV labeled with [^{3}H]leucine. Proteins of molecular weight 210K, 110K, 21.5K, 21K, and 20K were labeled with [^{3}H]fucose and correspond to unique EEV proteins detected by Coomassie brillant blue staining. Two proteins not observed after protein staining were detectable by glycoprotein labeling and had molecular weights of 89K and 42K. The 37K protein revealed by protein staining was not labeled by [^{3}H]fucose or [^{3}H]glucosamine. Protein staining and glycoprotein labeling thus revealed a total of eight EEV-specific proteins, seven of which were glycosylated. None of these proteins could be prelabeled with [^{3}H]leucine (gel not shown).

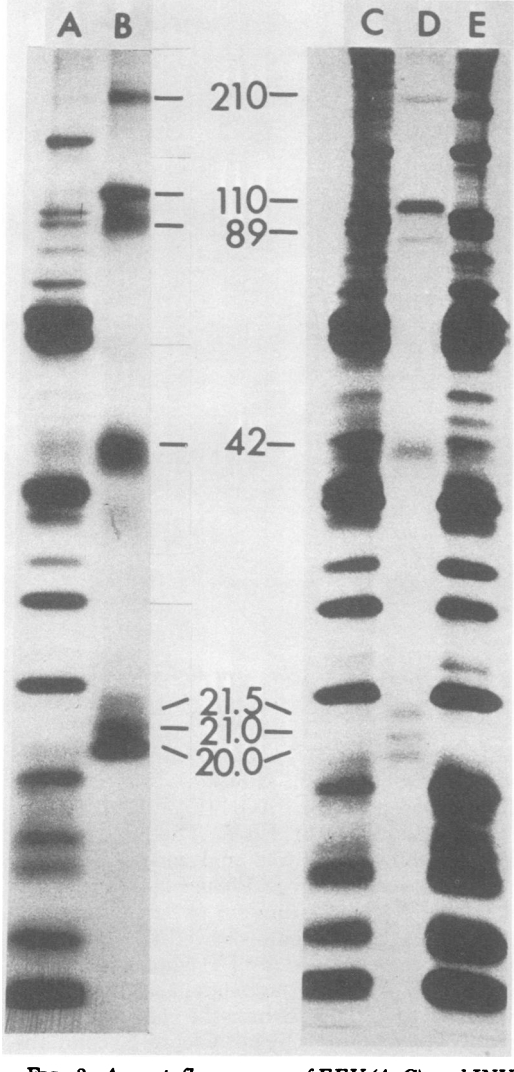

FIG. 3. *An autofluorogram of EEV (A, C) and INV (E) from HeLa cells labeled with [^{3}H]leucine compared with EEV from HeLa (B) and SIRC (D) labeled with [^{3}H]fucose.*

Characterization of EEV proteins after Brij-58 treatment. To determine the location of proteins unique to EEV, experiments were conducted to separate the envelope from EEV particles and to analyze separately the resultant envelope and particle fractions in PAGE. We employed the mild nonionic detergent Brij-58 to effect this separation. Figure 4A shows the coincidence of [^{14}C]thymidine and [^{3}H]fucose peaks of untreated purified EEV at a density of 1.23 to 1.24 g/ml. Brij-58 treatment of EEV followed by centrifugation in cesium chloride is depicted in Fig. 4B. This treatment resulted in a redistribution of the [^{14}C]thymidine and [^{3}H]-fucose. A peak containing only [^{14}C]thymidine appeared at a density of 1.27 g/ml, which corresponds to the density of INV (14). No envelopes were detected when these particles were examined by electron microscopy. A second peak containing only [^{3}H]fucose remained at the top of the gradient and was therefore assumed to represent envelope fragments from EEV. A similar result was obtained when EEV was Tween 80-ether treated; however, material was often lost at the water-ether interface, reducing the recovery rate.

Polypeptide analysis of untreated INV and EEV and the stripped virus particles and envelope fractions after Brij-58 treatment of EEV is shown in Fig. 5. The envelope fraction contained several minor proteins which were also present in INV and therefore do not represent envelope proteins. The EEV unique proteins that were completely removed by Brij-58 treatment had molecular weights of 210K, 110K, 21.5K, 21K, and 20K. The 89K and 42K proteins were not detected in the envelope fraction by protein staining. However, all seven glycoproteins were completely removed when scintillation autofluorograms of [^{3}H]fucose-labeled EEV were made after Brij-58 treatment (gel not shown). The 37K protein could also be removed from EEV. The comigrating INV protein was not removed from INV particles (see Fig. 6) and therefore presumably not from EEV.

Susceptibility of INV surface proteins to detergent, ether, and Pronase treatment. Two types of experiments were performed to characterize the surface proteins of INV particles. The first approach was to treat INV with nonionic detergents or Tween 80-ether (Fig. 6). Several of the INV proteins were represented in the supernatants after such treatment, but they varied greatly as to the quantity and species of protein released. Brij-58 did not release any INV protein to a significant degree. Both NP-40 and Cutscum removed all or the majority of proteins 30K and 27K. Treatment with Tween 80 followed by an ether extraction released a signifi-

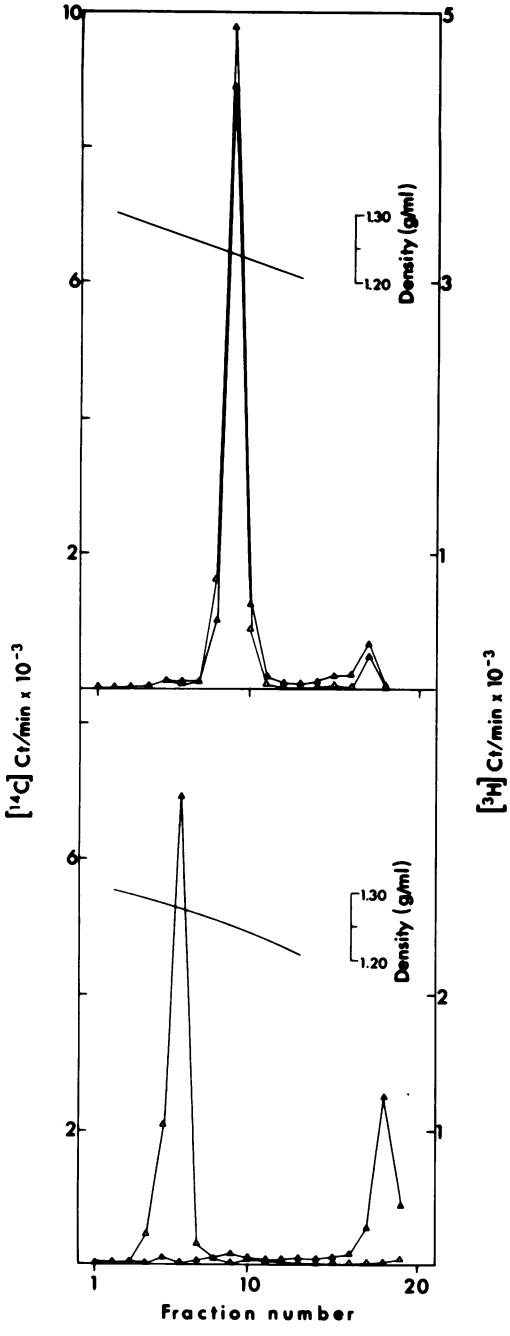

FIG. 4. *Cesium chloride gradients of untreated EEV (upper panel) and 1% Brij-58-treated EEV (lower panel) labeled with [^{14}C]thymidine (△) and [^{3}H]fucose (▲).*

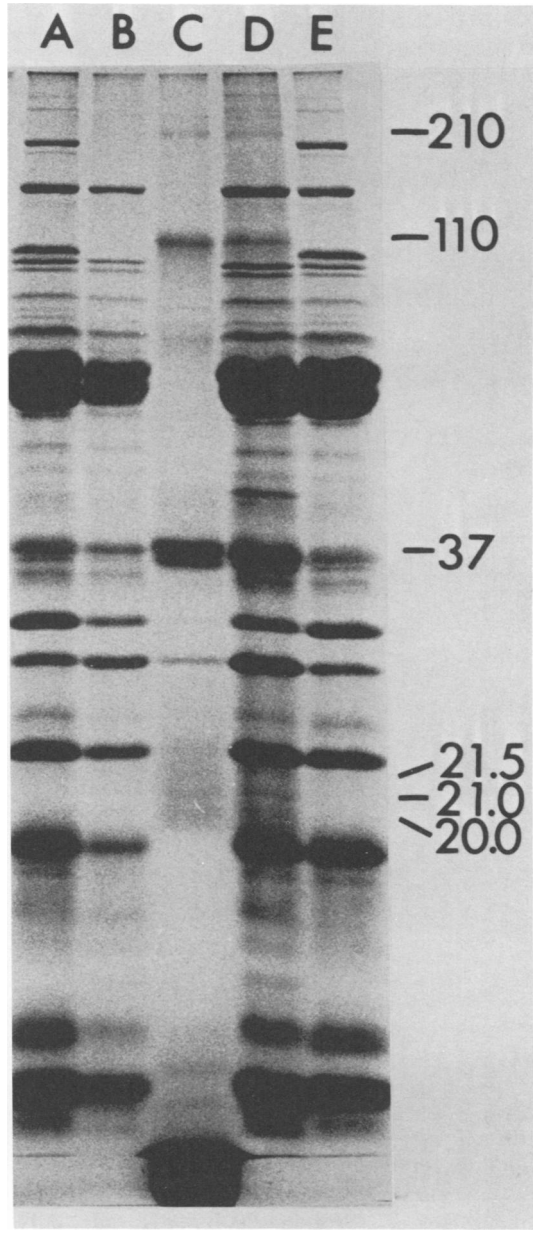

FIG. 5. *PAGE analysis of virus particles (B) and envelope fractions (C) derived from EEV treated with 1% Brij-58 and separated on cesium chloride as described in the text. Untreated INV (A and E) and untreated EEV (D) are included for comparison.*

cant proportion of proteins 200K and 95K. These four proteins are the same ones that are either absent or altered in concentration in EEV particles compared to INV particles. Proteins 65K and 13K, which were also radically altered in EEV in comparison with INV, were extractable by Tween 80-ether treatment, but the efficiency of removal varied from experiment to experiment. Figure 6 shows an experiment in which very little of the 65K and 13K proteins was removed. A second approach was to study the susceptibility of INV proteins to Pronase treat-

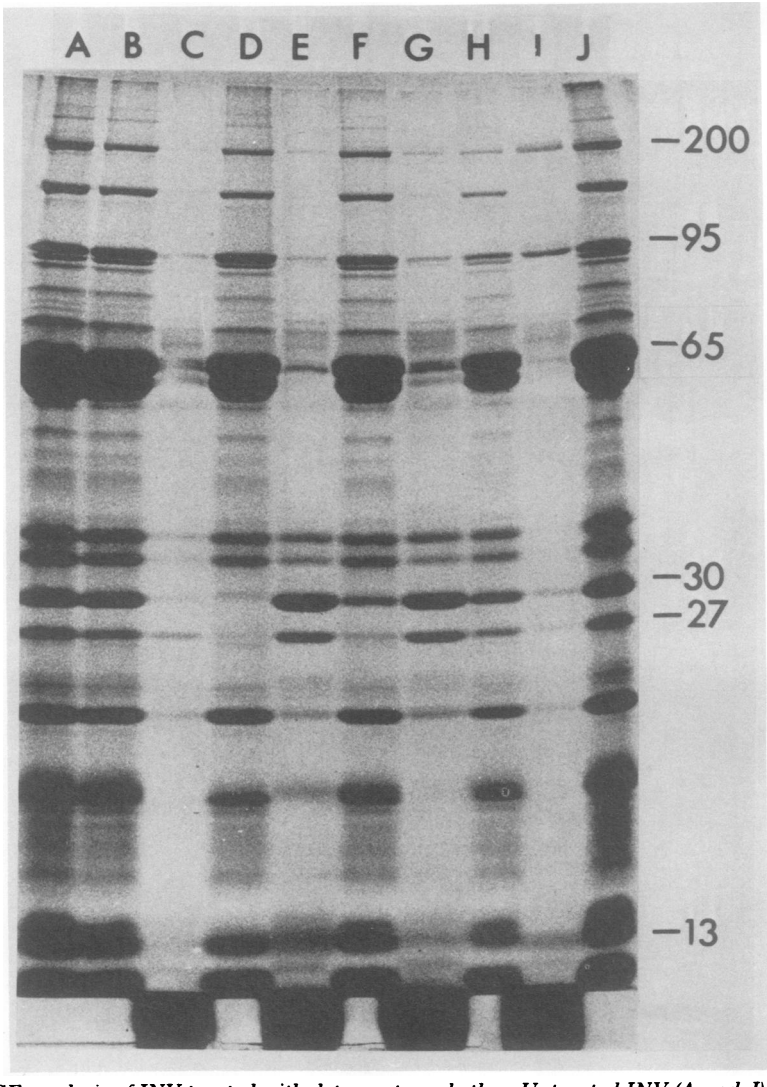

FIG. 6. *PAGE analysis of INV treated with detergents and ether. Untreated INV (A and J) is included for comparison. INV was treated and separated into particles and supernatant as described in the text: particles (B) and supernatant (C) after 1% Brij-58; particles (D) and supernatant (E) after 0.5% NP-40; particles (F) and supernatant (G) after 1% Cutscum; and particles (H) and supernatant (I) after Tween 80-ether extraction.*

ment (Fig. 7). Proteins 200K, 95K, 65K, 30K, 27K, and 13K were all sensitive to Pronase activity. No other proteins were significantly affected. Several new minor proteins and a major 24K protein absent from untreated INV and EEV appeared after Pronase treatment.

The effect of these various treatments on the protein composition of INV was also correlated to the biological function of infectivity (Table 1). Brij-58 did not significantly reduce infectivity, whereas Tween 80-ether inactivated 48% of the INV infectivity. Cutscum and NP-40 both reduced the infectivity by more than 95%. In contrast, Pronase treatment increased INV infectivity by 2.14 times. Electron microscopic examination showed that this increase reflects a further dispersal of virus particle aggregates not achieved by the vigorous sonic disruption of virus suspensions prior to the addition of Pronase.

DISCUSSION

The existence of extracellular vaccinia virions surrounded by an envelope not present on intracellular virions is well documented (1, 4, 14). The present study was directed at analyzing the protein structure of these EEV. Attainment of a highly purified EEV preparation was of para-

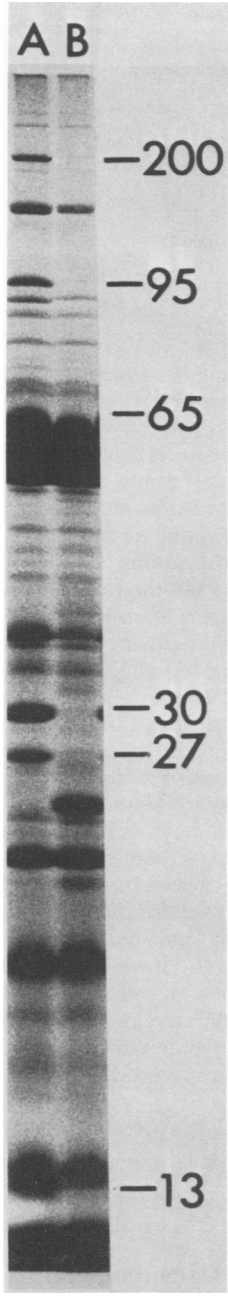

FIG. 7. *PAGE analysis of untreated INV (A) and INV treated with 10 μg of Pronase per ml for 60 min at 37°C (B).*

mount importance in the determination of the EEV protein composition, and was dependent on two factors. First, the cell line used for EEV cultivation greatly affected the possibilities for purification. HeLa cells released such large quantities of debris into the extracellular medium that we were unable to obtain EEV preparations of a sufficiently high purity. In contrast, the purification of EEV was facilitated by the low level of cell contaminants released by SIRC cells. Second, the method of purification affected the quality and quantity of EEV obtained. Equilibrium centrifugation in 30 to 60% continuous sucrose gradients, followed by equilibrium centrifugation in cesium chloride, was clearly superior to other centrifugation methods. The tenacious cellular protein actin was removed only by this purification scheme.

The analysis of the envelope proteins required a method for the separation of the envelope from the enclosed vaccinia particle. We have taken advantage of the relative insensitivity of vaccinia intracellular virus to Brij-58 treatment (18) to obtain an envelope-rich fraction. This treatment of EEV yields infectious naked particles and a solubilized envelope fraction that can be separated by cesium chloride centrifugation.

Examination of EEV and INV by PAGE revealed striking differences in their protein composition, which are summarized in Table 2. Eight proteins were unique to EEV. Seven of these proteins were glycoproteins and therefore presumably located on the envelope surface. The 37K protein was the only unglycosylated EEV unique protein. It may have a location and function analogous to the matrix proteins of several enveloped viruses. All eight proteins are presumed to be envelope or envelope-associated proteins, since they were selectively removed by Brij-58 treatment. Hemagglutinin is present in the envelope of EEV (14), but its relationship with these proteins is unknown. It is similarly not known whether they are the virus-specified proteins that appear in the membranes of infected cells (23), although there is a resemblance in the protein distribution. Further work is needed to investigate these similarities. Table 2 also shows the INV proteins that were either absent from EEV or altered in concentration compared to INV. These proteins are located at or near the surface of INV particles, since they were all sensitive to Pronase treatment and showed varying degrees of susceptibility to detergent and ether extraction. Our results using detergents are similar to previously published results (11, 17). These proteins are not necessary for vaccinia infectivity, since their removal by Pronase did not inactivate the virions. It is interesting that many of these INV protein alterations found in EEV from HeLa cells were already evident on SIRC cell-grown INV particles. The difference in INV preparations from HeLa and SIRC cells may represent a basic difference in the efficiency of the two cell lines to perform the INV surface protein alterations. It is also

TABLE 1. *Effect of detergents, ether, and Pronase on INV infectivity*[a]

Treatment	[^{3}H]thymidine (cpm/0.2 ml)	Infectivity (PFU/0.1 ml × 10^{-5})	Ratio of infectivity to [^{3}H]thymidine (PFU/cpm × 10^{-4})	Normalized	% Inactivation
Untreated	3,653	282	1.54	1.00	0
Brij-58, 1%, 10 min, 20°C	3,378	256	1.51	0.98	2
Tween 80-ether extraction	1,970	64	0.65	0.42	48
Cutscum, 1%, 30 min, 20°C	3,830	12	0.063	0.041	96
NP-40, 0.5%, 60 min, 37°C	4,266	3	0.014	0.009	99
Untreated		29		1.00	
Pronase, 10 μg/ml, 60 min, 37°C		62		2.14	

[a] 100 μg of INV in 1 ml of phosphate-buffered saline (pH 7.2) was treated with various agents as described in the table and the text. Four 0.2-ml samples were precipitated for the radioactivity determinations, and their mean is shown for each treatment. Infectivity was determined by plaque titration in triplicate.

TABLE 2. *Summary of properties for certain EEV proteins*

Proteins	Mol wt (×10^3)	Protein type[a]	Concn in EEV relative to INV
EEV unique	210	GP	
	110	GP	
	89	GP	
	42	GP	
	37	P	
	21.5	GP	
	21	GP	
	20	GP	
INV altered in EEV	200		Absent
	95		Absent
	65		Reduced
	30		Reduced
	27		Increased
	13		Absent

[a] GP, Glycoprotein; P, protein.

possible that high- and low-EEV-yielding vaccinia strains can differ in this respect.

The in vivo alterations of the INV surface structure are probably the result of enzymic cleavage. This processing may or may not release some cleavage products from the INV surface. It is noteworthy that no new non-envelope proteins were found in EEV. This would indicate that the particle-associated cleavage products must already be represented in the INV particle or comigrate with other INV proteins. The in vitro cleavage by Pronase provides very little information in this regard, since this enzyme completely removed the surface proteins and resulted in a new 24K protein not present after the in vivo cleavage.

In stationary cultures of vaccinia-infected cells, the only detectable extracellular virus even at 72 h postinfection (unpublished data) is in the enveloped form. The in vitro dissemination of vaccinia virus is due to the release of this extracellular virus (3). One conclusion from the results reported here is that this release of vaccinia virus from infected cells requires a processing of INV surface proteins and the acquisition of an envelope containing an additional set of unique virus-specified proteins.

The in vivo production of EEV containing unique proteins is of obvious immunological importance. The inefficacy of inactivated vaccinia vaccines composed of INV has been repeatedly demonstrated (reviewed in 4). This can be explained in light of our findings. The dissemination of infection can be mediated by either EEV or by EEV particles that may have lost their envelopes. The envelope of intact EEV particles would contain new antigenic specificities not recognized by antibodies directed against INV. The envelope would have the additional effect of masking the more internal particle antigens and preventing their reaction with INV-specific antibodies (1, 4). However, even if the masking envelope is lost, the processing of INV surface proteins during envelopment would result in a de-enveloped particle with an antigenic surface structure distinct from INV.

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