Two-Dimensional Polyacrylamide Gel Analysis of *Plodia interpunctella* Granulosis Virus†

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The structural polypeptides of purified *Plodia interpunctella* granulosis virus were analyzed by three different two-dimensional gel systems. Isoelectric focusing followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis allowed resolution of 53 acidic polypeptides in the enveloped nucleocapsid of the virus ranging in molecular weight from 97,300 to 8,000. Nine of these polypeptides were shown to be glycoproteins by the technique of radiolabeled lectin blotting. Separation of the granulin in this system allowed resolution of five species, all of which have identical tryptic peptide maps. This matrix protein was demonstrated to be a phosphoglycoprotein by radiolabeled lectin blotting and acid phosphatase dephosphorylation. Nonequilibrium pH gel electrophoresis followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis allowed resolution of the major basic protein of the virus, VP12, from a more acidic protein of the same molecular weight. Tryptic peptide analysis demonstrated that these two proteins were indeed different and acid urea gels followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis allowed localization of the acidic protein to the envelope and the basic protein to the nucleocapsid of the virus. Finally, probing of the separated envelope nucleocapsid proteins in both the isoelectric focusing and nonequilibrium pH gel electrophoresis two-dimensional systems after transfer to nitrocellulose with iodinated, purified viral proteins allowed further insight into reactions which may be important in the maintenance of the virion structure.

The granulosis (GV) and nuclear polyhedrosis viruses, members of the family *Baculoviridae*, are structurally complex, rod-shaped DNA viruses. Our laboratory has been investigating the granulosis virus of the Indian meal moth *Plodia interpunctella* (PiGV) for a number of years. This investigation has principally involved characterization of the viral structural proteins, the viral nucleic acid, and some of the enzymes critical to the replication of the virus in its insect host. One study carried out by Tweeten et al. (29) demonstrated by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) the presence of 19 structural polypeptides in the virus. The major polypeptide, granulin, was found to have a molecular weight of 28,000, while virions contained 18 polypeptides ranging in molecular weight from 12,600 to 97,300. Other investigators have also studied various GVs by one-dimensional electrophoretic methods (10, 25, 32). The granulins of all of the GVs have molecular weights similar to that of PiGV (molecular weight, 28,000 to 30,000). Additionally, the virions of the GVs have been reported to have from 12 to 19 structural polypeptides ranging in molecular weight from 98,000 to 12,000.

Recently, Singh et al. (23, 24) used two-dimensional gel systems to study the nuclear polyhedrosis viruses of *Autographa californica* and *Purthetria dispar*. Using isoelectric focusing (IEF) followed by SDS-PAGE, these investigators found 81 acidic polypeptides ranging in molecular weight from 13,500 to 86,000 in *A. californica* nuclear polyhedrosis virus and 95 acidic polypeptides ranging in molecular weight from 13,500 to 85,000 in *P. dispar* nuclear polyhedrosis virus (23). Additionally, resolution of 100 basic and acidic polypeptides ranging in molecular weight from 11,800 to 105,000 in *A. californica* nuclear polyhedrosis virus

was possible by using the nonequilibrium pH gel electrophoresis (NepHGE) SDS-PAGE two-dimensional system (24). These powerful two-dimensional systems have the potential to reveal a great deal of information concerning the structural polypeptides of baculoviruses. The present study was designed to examine the structural polypeptides of *P. interpunctella* to further characterize the structural proteins of this virus by using the techniques of two-dimensional gel electrophoresis, peptide mapping, and protein (Western) blotting. Both lectins and viral proteins were used as probes in these studies, which allowed us to understand more clearly the complex nature of this virus.

MATERIALS AND METHODS

Production and purification of GV. GV was produced in a laboratory colony of *P. interpunctella* as previously described (26). Early-third-instar larvae were infected per os with GV, and the virus was purified by differential centrifugation, treatment with 1% deoxycholate, and velocity sedimentation in sucrose gradients (26, 27).

Isolation and purification of viral components. Granulin, enveloped nucleocapsids (ENCs), and capsids were isolated and purified as previously described (29). Envelope material was removed from the tops of glycerol gradients during nucleocapsid purification. This material was concentrated and filtered twice through Amicon YM-5 membranes and is a partially purified preparation enriched in membrane proteins. The basic protein, VP12, was acid extracted from nucleocapsids or ENCs as previously described (28).

Solubilization of samples. Virus preparations were lyophilized to dryness and suspended in an appropriate buffer for electrophoresis in the first dimension. When the first dimension was either IEF or NEpHGE the solubilization buffer was lysis buffer (17) modified to contain 4% Nonidet P-40 (23). The virus components were subjected to five cycles of freezing, thawing, and vigorous vortexing in this buffer prior to electrophoresis. When acid urea gels were run in the first

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FIG. 1. pH gradient for IEF gels. The zero point on the abscissa represents the basic (top) end of the gel.

dimension, the solubilization buffer was that described by Panyim and Chalkley (21). Again the viral components were subjected to five cycles of freezing, thawing, and vortexing in this buffer prior to electrophoresis.

Iodination of viral components. Purified viral components were frozen and thawed three times in distilled water and then iodinated with ^{125}I by the chloramine-T method (9). Unlabeled tyrosine was used to stop the reaction, and free iodine was removed by passing the sample through a column of G-25 Sephadex. The labeled virus was then concentrated to dryness and suspended as described above.

IEF. First-dimension IEF was carried out as described by O'Farrell (17) with the following modifications of Singh et al. (23). The focusing gel mixture contained 9.5 M urea, 4% acrylamide, 2% Nonidet P-40, and 2% ampholines consisting of pHs 4 to 6, 6 to 8, and 3.5 to 10 at a ratio of 2:2:1 (1). Ammonium persulfate and N,N,N',N' -tetramethylethylenediamine were used to initiate polymerization. Virus samples (25 to 100 μg of protein) were applied at the basic end of the gel following pre-electrophoresis. Electrophoresis was carried out toward the anode for 6,000 V-h. Focused gels were equilibrated for SDS-PAGE by gentle agitation in SDS equilibration buffer (17) for 30 min and stored wrapped in Parafilm at -20°C for a maximum of 1 week. We monitored the pH gradient with a pH meter after slicing a focused gel into 5-mm sections and incubating these sections overnight in 1 ml of distilled, deionized water. The gradient usually ran between pH 3.5 and 7.0 (Fig. 1).

Nonequilibrium pH gel electrophoresis. First-dimension NEpHGE was carried out as described by O'Farrell et al.

(19) with the following modifications described by Singh et al. (24). The gel mixture contained 9.5 M urea, 4% acrylamide, 2% Nonidet P-40, and 2% ampholines consisting of pHs 7 to 9 and 3.5 to 10 at a 2:1 ratio. Ammonium persulfate and N,N,N',N' -tetramethylethylenediamine were used to initiate polymerization. Virus samples (25 to 100 μg of protein) were applied to the acid end of the gel and electrophoresed toward the cathode for 2,000 V-h. Gels were equilibrated as described above. The pH gradient of these gels ranged from 4.0 to 8.0 and was slightly unstable at the basic end (Fig. 2).

Acid urea gels. First-dimension acid urea gels were prepared as described by Panyim and Chalkley (21) to give a final pH of 3.2. Pre-electrophoresis was carried out at 1 mA per tube for 2 h. After removing the top buffer, we loaded the samples, and electrophoresis was carried out at 1 mA per tube until the methyl green dye marker eluted. Gels were equilibrated as described above.

SDS-PAGE and autoradiography. All separations were performed on 15% SDS-polyacrylamide gels as described by Laemmli (15). Gels measured 140 by 110 by 1.5 mm with a 10-mm stacking gel. The electrode buffer was 0.05 M Tris-0.38 M glycine-0.1% SDS, pH 8.7. The first-dimension tube gel was either placed horizontally on top of the stacking gel (IEF and acid urea) or sliced and placed in wells made in the stacking gel (NEpHGE). Agarose dissolved in distilled water (0.5%) was used to hold the gel in place. Purified GV ENC was added to a well in the agarose (IEF and acid urea) or a well in the stacking gel (NEpHGE) to serve as molecular weight markers. The gels were electrophoresed at 25 mA per

FIG. 2. pH gradient for nonequilibrium pH gels. The zero point on the abscissa represents the acidic (top) end of the gel.

FIG. 3. Two-dimensional analysis of PiGV granulin. IEF in the horizontal direction (first dimension) followed by SDS-PAGE in the vertical direction (second dimension). (A) Diagram of the gel in panel B. The five species of granulin separated by IEF were named A to E starting from the basic (top) end of the gel as indicated. (B) Coomassie blue-stained gel of PiGV granulin separated in two dimensions. Both the dimer (molecular weight, 74,000) and many breakdown products (molecular weights, 27,000 to 8,000) were stained in addition to the five major species (molecular weight, 28,000). (C) Granulin in species probed with *U. europaeus* agglutinin. Molecular weights (10^3) are on the ordinates of all three panels.

gel until the bromphenol blue dye marker which was added to the cathode buffer had migrated through the gel (usually 4.5 to 5 h). Gels were stained with 0.1% Coomassie blue and destained with several changes of methanol-acetic acid-water solutions (4). Destained gels were photographed, dried, and, if labeled virus was used, subjected to autoradiography with Kodak XAR-5, X-ray film. The film was processed with Kodak GBX developer and Kodak fixer.

Protein (Western) blotting. Proteins were electrophoretically transferred to nitrocellulose sheets for 4 h at 5 W as described by Bittner et al. (6). After transfer, the nitrocellulose sheets were used for lectin or protein binding as described below.

Lectin binding. Nitrocellulose sheets that were to be probed with ^{125}I -labeled lectins were incubated in a buffer containing 2% polyvinylpyrrolidone as previously described (22). Lectins used in this study were concanavalin agglutinin, *Dolichos biflorus* agglutinin, *Limulus polyphemus* agglutinin, and *Ulex europaeus* agglutinin. Buffers used were those recommended by the manufacturer. The lectins were labeled and the blots were probed as previously described

(22). Autoradiography was used to detect the proteins having carbohydrate moieties.

Protein binding. Nitrocellulose sheets that were to be probed with ^{125}I -labeled capsids or ^{125}I -labeled acid-extracted VP12 were incubated in TDN buffer (0.01 M Tris, 0.002 M disodium EDTA $2\text{H}_2\text{O}$, 0.05 M NaCl, pH 7.5) containing 4% bovine serum albumin and 0.05% Tween 20 overnight at room temperature. Capsids or VP12, iodinated as described above, were added to 50 ml of this buffer (2×10^7 cpm), and the blocked nitrocellulose sheet was incubated in this solution overnight at room temperature. Excess label was removed by extensive rinsing in TDN buffer containing 0.05% Tween 20. Autoradiography was used to detect the proteins which bound to either of these probes.

Peptide mapping. Sections of dried SDS-polyacrylamide-gel-separated proteins were excised and digested for 16 h in buffer containing 0.05 M NH_4HCO_3 , pH 8.0, with 1.5 M dithiothreitol and 50 μg of L-1-tosylamide-2-phenylethyl chloromethyl ketone-trypsin per ml at 37°C . Supernatant fluids were lyophilized to dryness and redissolved in 5 μl of electrophoresis buffer (acetic acid-formic acid-water, 3:1:16). The sample was spotted in a corner of a 10 cm by 10 cm precoated cellulose thin-layer chromatography plate (MC/B Manufacturing Chemists, Inc., Cincinnati, Ohio; catalog no. 5611) and electrophoresed for 18 to 20 min at 500 V. A solution containing orange G (2%) and acid fuchsin (1%) dissolved in electrophoresis buffer was spotted opposite the sample and used to track migration in this dimension. After the plates had dried, ascending solvent chromatography in a buffer containing *n*-butanol-pyridine-acetic acid-water (32.5:25:5:20) was performed in the second dimension (2, 12). Autoradiography was used to detect the peptides.

Dephosphorylation of viral proteins. Purified preparations of the granulin protein were treated in vitro with either 10 U of alkaline phosphatase VII-S (Sigma Chemical Co., St. Louis, Mo.) or 2 U of Potato Acid Phosphatase Grade I (Sigma) for 1 h at 37°C . Alkaline phosphatase dephosphorylation was carried out in 20 mM Tris hydrochloride buffer, pH 8.0, which contained 5 mM MgCl and several protease inhibitors (5). These included aprotinin, phenylmethylsulfonyl fluoride, *N*-*p*-tosyl-L-lysine chloromethyl ketone, L-1-tosylamide-2-phenylethyl chloromethyl ketone, and leupeptin, all at a concentration of 10 $\mu\text{g}/\text{ml}$. Acid phosphatase dephosphorylation was carried out in a 50 mM acetate buffer, pH 5.6, containing these same protease inhibitors (5). The reactions were stopped by lyophilization, and the samples were suspended in IEF lysis buffer and handled as previously described. Controls included untreated virus samples as well as samples reacted with heat-inactivated (boiled) enzyme preparations.

RESULTS

Granulin polypeptides. Granulin protein isolated from protease-active GV and treated as described above was first electrophoresed on IEF tube gels and then on SDS-polyacrylamide slab gels. After staining with Coomassie brilliant blue, several polypeptides were visible. As previously reported, the major peptide had a molecular weight of 28,000 (28). Minor peptides ranging in molecular weight from 10,000 to 74,000 were also observed. These were believed to be either breakdown products of the granulin due to the active protease or dimers of the 28,000-molecular-weight species. When the protease was heat inactivated, the smaller peptides were no longer visible (data not shown); however, two larger peptides (molecular weights 66,000 and 74,000)

FIG. 4. Tryptic peptide maps of granulin species. (A) Map of species A. (B) Map of species C. (C) Map of species E. (D) Consensus map of all five species (species B and D were analyzed, but owing to space limitations the actual data are not shown). The spot which is dashed is much reduced in species E; however, it is visible on long exposure of the map. The directions of electrophoresis (E) and chromatography (C) are indicated.

FIG. 5. IEF analysis of granulin and phosphatase-treated granulin. Symbols: ■, granulin, untreated; ●, granulin treated with alkaline phosphatase; ○, granulin treated with acid phosphatase. ○, pH gradient.

FIG. 6. Two-dimensional analysis of PiGV ENC. IEF in the horizontal direction (first dimension), SDS-PAGE in the vertical direction (second dimension). (A) Diagram of the two-dimensional gel shown in B. Molecular weights of three virus polypeptides are indicated as calculated molecular weight (10^3). The three species of VP31 are indicated by solid lines, and a fourth possible species is indicated by a dashed line. (B) Coomassie blue-stained gel of PiGV ENC separated in two dimensions. The three major species of VP31 were named a, b, and c starting from the basic (top) end of the gel (Fig. 7D). (C) An autoradiogram of ENC probed with ^{125}I -*Dolichos biflorus* agglutinin showing nine glycoproteins in the molecular weight range of 86,000 to 12,600 as designated on the ordinate. Molecular weights (10^3) are on the ordinates of all three panels.

could still be observed. This is consistent with a previous report (29).

Two-dimensional analysis revealed the presence of five species of granulin having a molecular weight of 28,000 (Fig. 3A and B). The pIs of these species ranged from 5.0 to 5.6. Tryptic peptide mapping analysis confirmed that these five species were indeed the same, thereby suggesting that the variation in pI was due to some form of posttranslational modification (Fig. 4A to D; only three actual maps are shown owing to space limitations; all five maps were identical).

Two modifications common to proteins were examined. First, by probing nitrocellulose sheets of transferred gels with ^{125}I -lectins it was determined that the granulin was modified by glycosylation. Two of the four lectins assayed bound to the granulin on the nitrocellulose sheet (Fig. 3C). These lectins were *V. europaeus* and *D. biflorus* agglutinins, which have specificity for L-fucose and *n*-acetyl-D-galactosamine residues, respectively. The other two lectins we tested, *L. polyphemus* agglutinin and concanavalin A agglutinin, which bind sialic acid and mannose residues,

FIG. 7. Two-dimensional gel and tryptic peptide analysis of PiGV ENC. (A, B, and C) Tryptic peptide maps of the three major species of VP31, a, b, and c, respectively (Fig. 6B). (D) Autoradiography of a two-dimensional gel of ^{125}I -labeled PiGV ENC. The species of VP31, a, b, and c (as defined on IEF), are indicated, as well as the two different proteins (e and f) migrating in the region of the gel corresponding to a molecular weight of 12,000. Molecular weights (10^3) are indicated on the ordinate. Brackets indicate protein-protein interactions between VP31 and both the acidic and basic VP12 proteins. (E and F) Tryptic peptide maps of the proteins migrating at 12,000. (E) Map of the acidic VP12 labeled e. (F) Map of the basic VP12 labeled f. The directions of NEpHGE, SDS-PAGE, electrophoresis (E), and chromatography (C) are indicated.

respectively, were unable to bind to the granulin on the nitrocellulose.

Glycosylation, however, does not seem to be solely responsible for the differences in the pIs of the five species of granulin, as the dimers and the breakdown products, as well as all five of the major spots bound the lectins (Fig. 3C). Tween et al. (29) previously reported the presence of low-level phosphate moieties on the granular protein following microinjection of $^{32}\text{P}_i$ into *Plodia*-infected larvae. Therefore, phosphorylation was also investigated. Because this virus has not yet been adapted to tissue culture, the traditional method of looking at protein phosphorylation by infecting cells with virus in media containing $^{32}\text{P}_i$ was not possible. Phosphorylation was therefore assayed indirectly with alkaline and acid phosphatases. By dephosphorylating the protein in vitro and monitoring the shift in pI from more acidic phosphorylated species to more basic dephosphorylated species on IEF gels, we determined that granulin is indeed a phosphorylated protein. The acid phosphatase caused a shift in pI from the normal range (5.0 to 5.6) to a more basic range (5.5 to 6.5) (Fig. 5). After treatment with this enzyme three peaks were observed, suggesting the possibility that the phosphate moiety may be cryptic in nature and not available for complete enzymatic dephosphorylation. When the acid phosphatase was inactivated by boiling, no shift in pI was observed (data not shown). It was also demonstrated that alkaline phosphatase had no activity in dephosphorylating granulin (Fig. 5).

Virion polypeptides. As has been recently reported for two different nuclear polyhedrosis viruses (23), analysis of viral proteins by two-dimensional gel electrophoresis (IEF-SDS-PAGE) allows visualization of many more polypeptides than can be identified by one-dimensional gel systems. This is also true in the case of PiGV. Fifty-three polypeptides were observed in the ENCs of the GV by this technique (Fig. 6A

and B), whereas only 15 proteins were separable by the one-dimensional method (29).

One type of protein modification, glycosylation, was investigated by this two-dimensional electrophoresis system in conjunction with Western transfer of protein to nitrocellulose sheets and probing with iodinated lectins. Using the probe specific for proteins which are modified with *n*-acetyl-D-galactosamine moieties (*D. biflorus* agglutinin), we detected several proteins in the virion which were glycosylated (Fig. 6C). In addition to those reported previously (21), three high-molecular-weight proteins (molecular weights, 88,600, 63,700, and 44,900) were observed in the virion. These additional proteins are visible in this two-dimensional system owing to the fact that large quantities of viral protein could be loaded onto the gel. This brings the number of glycoproteins observed in PiGV to 10. Of these, nine are associated with the ENC and have molecular weights of 88,600, 63,700, 44,900, 39,700, 31,000 (four species), 29,900, 26,600, 17,600, and 12,600. The granulin (28,000) is also glycosylated.

It was observed in the two-dimensional systems (both IEF and NEpHGE followed by SDS-PAGE) that the major capsid protein of the virus (molecular weight, 31,000) seemed to be separable into four different species (Fig. 6A and B and 7D). Tryptic peptide analysis of the three major species showed that all of them had the same map (Fig. 7A, B, and C), suggesting that the difference in migration between the species might be due to posttranslational modification. The fourth species (Fig. 6A, dashed line) was consistently too low in radioactivity to allow for tryptic peptide mapping analysis. Earlier investigations by our laboratory revealed that VP31 is a glycosylated (22) but not a phosphorylated (29) protein. The studies with the NEpHGE-SDS-PAGE system also suggested that interactions between the VP31 and other viral proteins might

FIG. 8. Assignment of 12,600-molecular-weight proteins to specific viral components by two-dimensional analysis. Acid urea gel electrophoresis in the horizontal direction (first dimension) and SDS-PAGE in the vertical direction (second dimension). Panels: (A) ENC; B, envelopes; C, nucleocapsid; D, acid-extracted nucleocapsid. The VP12 basic protein (b) migrated into the gel and was therefore located to the right of the VP12 acidic protein (a), which did not migrate as far into the gel. The positions of proteins migrating at 12,600 molecular weight are indicated on the ordinates (12).

contribute to this difference in migration. This is discussed in more detail below.

Both NEpHGE and acid urea gels were used in the first dimension followed by SDS-PAGE to investigate the polypeptides with a molecular weight of 12,600. Previously our laboratory has reported on a protein of this size which is very basic in character and associated with the viral nucleic acid (28). It is also the major acceptor protein for phosphorylation by a capsid-associated protein kinase (30, 31) and is glycosylated (22). Typically, this protein is purified from the virus by acid extraction of the nucleocapsids (28). Using the two dimensional systems, we identified another species in this same molecular weight range which was not detectable by the one-dimensional SDS-PAGE system. This protein was more acidic in nature, as it would not migrate into either NEpHGE (Fig. 7D, label e) or acid urea (Fig. 8A and B) gels. Its location was determined to be within the viral envelope because it was present in both ENC and envelope preparations (Fig. 8A and B) but was absent in nucleocapsid preparations (Fig. 8C and D). This protein, unlike the basic VP12, is not glycosylated (Fig. 6C). Tryptic peptide analysis demonstrated that these two species have different amino acid sequences (Fig. 7E and F).

Both of these 12,600-molecular-weight proteins were shown to interact with the major capsid protein, VP31, by the technique of radiolabeled protein blotting. The basic VP12 reacts with the capsid protein, VP31, when the VP31 is denatured (Fig. 9C), whereas the acidic VP12 reacts with the VP31 only when the VP31 is in its native (capsid) state (Fig. 9B). These results are consistent with the fact that the basic VP12 is associated with the DNA located within the capsid and would probably react with a different site on the capsid protein molecule than would the acidic VP12, which is an envelope protein.

DISCUSSION

The results of the experiments described in this report reveal that the structural organization of the GV is indeed

complex. First, the evidence indicates that the major viral protein, granulin, is modified posttranslationally by both phosphate and carbohydrate moieties. These modifications cause the appearance of five species which differ slightly in pI when the protein is examined by two-dimensional gel electrophoresis. Additionally, the major capsid protein of the virus, VP31, appears to be modified by carbohydrate residues and is separable into at least three species by two-dimensional electrophoresis. This phenomenon has been observed in several other viral systems, including the papovaviruses (7, 18), mouse c-type endogenous viruses (11), herpes simplex virus type 1 (13) vaccinia virus (20), and vesicular stomatitis virus (14). Furthermore, recent studies on the polyomavirus major capsid protein, VP1, which is separable into six species by two-dimensional electrophoresis (7), have demonstrated that the modifications contributed by the host which are responsible for the differences in pI also alter the function of the protein (2, 3, 7-9, 16, 33). This is important, as it demonstrates a mechanism by which a single polypeptide encoded by the virus can play several roles in the life cycle of the virus. In the case of polyomavirus, these roles vary from structural support and organization to attachment of the virion to the cell surface. It is possible that modification of the baculovirus proteins also allows these proteins to assume a variety of roles in the virus life cycle.

Second, two-dimensional electrophoresis has allowed the resolution of over 50 acidic polypeptides and several basic polypeptides in the ENC of this GV. As reported by Singh et al. (23), assuming an average gene size of 0.8×10^6 daltons, the large genome of this and other baculoviruses might consist of 75 to 100 genes. As demonstrated herein, not all of these polypeptides are independently coded for (i.e., three of these spots migrating at 31,000 were shown to have identical peptide maps, and the granulin is separable into five distinct spots, all of which are identical in amino acid sequence as determined by mapping); however, it is probable that the genome codes for more structural proteins than the 18 previously reported by our laboratory (29). For instance, in

FIG. 9. Two-dimensional gel analysis of PiGV ENC to identify protein-protein interactions. The directions of NEpHGE and SDS-PAGE are indicated. (A) Coomassie-stained two-dimensional gel. (B) Nitrocellulose sheet of a transferred two-dimensional gel probed with ^{125}I -labeled PiGV capsids. Binding of the capsids to acidic VP12 is indicated (12 arrow). (C) Nitrocellulose sheet of a transferred two-dimensional gel probed with ^{125}I -labeled basic VP12. Binding of this protein to the major capsid protein, VP31, is indicated (31 arrow). Numbers on the ordinates are also the molecular weights of the proteins (10^3).

the molecular weight range of 45,000 to 25,000 the two-dimensional IEF-SDS-PAGE system allows for the resolution of 36 polypeptides in contrast to the 7 reported for the one-dimensional SDS-PAGE system. Some of these may be novel, unique proteins. A particular example of this was demonstrated in the case of the proteins which migrate at 12,600 in one-dimensional SDS-PAGE. Whereas only one protein was observed in the one-dimensional system, both the NEpHGE and acid urea two-dimensional systems allowed resolution of two distinct proteins; one a basic DNA-binding protein and one an acidic envelope protein, both of which have this low molecular weight. Obviously, these two-dimensional systems are powerful tools for the study of the structural proteins of these complex viruses.

Finally, these studies demonstrated that there may be interactions between the major capsid protein (VP31) and both the DNA-protein core and the viral envelope. Acid-extracted VP12, the DNA-binding protein, in addition to binding to itself, is capable of binding to the major capsid protein after this protein has been denatured by SDS-PAGE. It is possible that denaturing the protein allows internal capsid binding sites to be exposed, thereby revealing an interaction which occurs between these two proteins in the intact virion. Intact capsids, in addition to binding denatured

VP31, can also bind to the acidic VP12 molecule of the viral envelope. Further evidence for interaction between these viral proteins could be seen when purified preparations of this virus were subjected to the various treatments involved in two-dimensional gel electrophoresis. Though the majority of the protein migrated in the nondenaturing first dimension according to its own charge, trace amounts migrated with the other protein to which it bound. This was particularly evident in the NEpHGE-SDS-PAGE system (Fig. 7D, brackets). It seems reasonable that the stability of the viral particle could be increased by protein-protein interactions not only within the capsid but between the core, the capsid, the envelope, and the matrix.

We feel that continued study of the structural proteins of the occluded form of this GV in terms of the functions of these proteins, their modifications, and their interactions by these powerful two-dimensional methods will be important in the understanding of how this virus reacts with host cells. Also, if the budded form of this virus can be isolated, comparison of the proteins of these two forms of the virus by two-dimensional techniques may reveal the subtle difference(s) which accounts for the inability of the occluded form to infect cells in tissue culture.

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