

Structural Polypeptides of the Granulosis Virus of *Plodia interpunctella*†

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Techniques were developed for the isolation and purification of three structural components of *Plodia interpunctella* granulosis virus: granulin, enveloped nucleocapsids, and nucleocapsids. The polypeptide composition and distribution of protein in each viral component were determined by sodium dodecyl sulfate discontinuous and gradient polyacrylamide slab gel electrophoresis. Enveloped nucleocapsids consisted of 15 structural proteins ranging in molecular weight from 12,600 to 97,300. Five of these proteins, having approximate molecular weights of 17,800, 39,700, 42,400, 48,200, and 97,300, were identified as envelope proteins by surface radioiodination of the enveloped nucleocapsids. Present in purified nucleocapsids were eight polypeptides. The predominant proteins in this structural component had molecular weights of 12,500 and 31,000. Whereas no evidence of polypeptide glycosylation was obtained, six of the viral proteins were observed to be phosphorylated.

Granulosis and nuclear polyhedrosis viruses are structurally complex viruses belonging to the family Baculoviridae. They infect a number of lepidopteran insects, causing disease and larval death in infected populations. Because of their effectiveness, the baculoviruses are being considered for use as biological insecticides for control of their insect hosts. As a result, these viruses have acquired scientific interest, and investigators have begun to obtain information on their molecular properties. Such knowledge is needed for developing methods of virus identification and detection and for determining their effects at the cellular level on nontarget species.

The basic structural unit of the granulosis virus (GV) and nuclear polyhedrosis virus (NPV) is a high-molecular-weight, covalently closed supercoiled DNA packaged within a rod-shaped capsid. The nucleocapsid is surrounded by a lipid envelope, and deposited on the outer surface of the envelope is a thick matrix of protein (1, 10, 21, 24). In NPV, several enveloped nucleocapsids are embedded within a protein crystal. This feature distinguishes it from GV, in which the enveloped nucleocapsids are singly occluded.

Identification of the baculoviruses based on characteristics of their matrix proteins has been shown to be inadequate. These proteins all have molecular weights of 26,000 to 30,000 (6, 11, 16, 22) and demonstrate serological cross-reactivity

(8, 11, 14). On the other hand, both qualitative and quantitative differences have been observed in the protein composition of the enveloped nucleocapsids and nucleocapsids from various NPV isolates (7, 11, 22).

Comparisons of GV, based on polypeptide composition, cannot be as readily made. Aside from extensive characterization of granulin, the major component of the GV protein matrix, the only structural proteins that have been examined are those of the enveloped nucleocapsids from the GVs of *Pieris brassicae* (5), *Trichoplusia ni*, and *Spodoptera frugiperda* (22). Like those of NPV, they consisted of 12 to 18 polypeptides ranging in molecular weight from 12,000 to 90,000. Information on the isolation and characterization of GV nucleocapsids is available only for the virus which infects *P. brassicae* (5).

Our laboratory is investigating the molecular biology of the GV of the Indian meal moth *Plodia interpunctella*. To analyze the structural polypeptide composition of this virus, we developed biophysical methods for its separation into three structural components: granulin, enveloped nucleocapsids, and nucleocapsids. These methods, along with the polypeptide composition and distribution of protein in each viral component, are reported in this paper. Other studies on NPV and GV have not provided direct evidence that specific viral polypeptides are constituents of the viral envelope. In the present work, radioiodination which specifically labels

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virus surface proteins was used to identify and localize the GV envelope polypeptides. Modifications of viral polypeptides by glycosylation and phosphorylation were also investigated.

MATERIALS AND METHODS

Production and purification of GV. GV was produced in a laboratory colony of *P. interpunctella* reared as previously described (23). 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 (23, 25).

Isolation of the protein matrix. The protein matrix was solubilized by incubating purified GV for 10 min at 37°C in 0.05 M sodium carbonate–0.05 M NaCl, pH 10.6. To isolate the matrix proteins in nondegraded form, the GV was heated before carbonate treatment for 30 min at 70°C to inactivate endogenous protease activity (25). The dissociated virus was layered on 30 to 70% glycerol (vol/vol, in 0.01 M Tris-hydrochloride, pH 7.5) gradients which were centrifuged at 25,000 rpm (SW 41 rotor) for 30 min at 10°C. The matrix proteins were recovered from the top of the gradient and concentrated in a model 12 Amicon ultrafiltration cell with a UM-10 membrane.

Isolation of enveloped nucleocapsids. Purified GV was incubated in 0.05 M sodium carbonate–0.05 M NaCl, pH 10.6, for 5 min at room temperature. The dissociated virus was layered on 30 to 70% glycerol (vol/vol, in 0.01 M Tris-hydrochloride, pH 7.5) gradients which were centrifuged at 25,000 rpm (SW 41 rotor) for 1 h at 10°C. The band of enveloped nucleocapsids was recovered from the gradients, diluted with 0.01 M Tris-hydrochloride, pH 7.5, and centrifuged at 25,000 rpm (SW 41 rotor) for 1 h to remove the glycerol. The enveloped nucleocapsids were stored in 0.01 M Tris-hydrochloride, pH 7.5, at 4°C.

Isolation of nucleocapsids. Purified enveloped nucleocapsids were incubated in 1% (vol/vol, in 0.01 M Tris-hydrochloride, pH 8.5) Nonidet P-40 (NP-40; Shell Chemical Co.) for 30 min with stirring at room temperature. The nucleocapsids were separated from the solubilized envelope proteins by sedimentation on 30 to 70% glycerol (vol/vol, in 0.01 M Tris-hydrochloride, pH 8.5) gradients centrifuged at 30,000 rpm (SW 41 rotor) for 1 h. The band of nucleocapsids was diluted with 0.01 M Tris-hydrochloride, pH 8.5, and centrifuged at 25,000 rpm (SW 41 rotor) for 1 h to remove glycerol. The nucleocapsids were stored in 0.01 M Tris-hydrochloride, pH 7.8, at 4°C.

Radiolabeling of GV in vivo. Radioactively labeled GV was produced in vivo by injection of 1 µl (0.1 to 0.5 µCi) of isotope into larvae at 96 and 120 h after infection. The isotopes used included [³H]thymidine (71 Ci/mmol, Schwarz/Mann), ¹⁴C-amino acid mixture (Schwarz/Mann), and [³²P]orthophosphoric acid (New England Nuclear). GV was purified from injected larvae 8 days after infection.

Iodination of enveloped nucleocapsids. Preparations of purified enveloped nucleocapsids containing 250 µg of protein in 100 µl of 0.01 M Tris-hydrochloride, pH 7.5, were mixed with 50 µl of 0.2 M phosphate buffer, pH 7.2, 0.5 mCi of [¹²⁵I]iodine (ICN Chemical and Radioisotope Division), 25 µl (0.5 mg) of Enzy-

mobeat reagent, and 25 µl of 1% β-glucose. After incubation at room temperature for 30 min, the Enzymobeads were removed by centrifugation at 250 × g for 5 min. Unreacted iodine was removed from the enveloped nucleocapsids by centrifuging the preparation on a 30 to 70% glycerol gradient for 1 h at 25,000 rpm (SW 41 rotor). The visible band of enveloped nucleocapsids was recovered and dialyzed for 24 h against 0.01 M Tris-hydrochloride, pH 7.5.

Glycosylation of viral components. To label galactosyl residues, a mixture consisting of 250 µg of virus (granulin, enveloped nucleocapsids, or nucleocapsids), 12 U of neuraminidase (Calbiochem), and 25 U of galactose oxidase (Sigma Chemical Co.) in 200 µl of 0.02 M phosphate buffer, pH 7.2, and 0.1 M NaCl was incubated for 1 h at 37°C. Then, 500 µCi of NaB[³H], (New England Nuclear) in 20 µl of 0.01 N NaOH was added, and the mixture was incubated at room temperature for 30 min. The viral preparations were then repurified by sedimentation on glycerol gradients. Sendai virus was labeled in a similar manner. Controls consisted of viral preparations that received no enzyme treatment. The viral proteins were then resolved by polyacrylamide slab gel electrophoresis and tritium-containing bands were detected by fluorography.

Electron microscopy of viral components. Samples of enveloped nucleocapsids and nucleocapsids were placed on Formvar-coated grids and negatively stained with 1% phosphotungstic acid, pH 7.0. Grids were examined with a Philips EM 201 at 60 kV.

Polyacrylamide gel electrophoresis of viral polypeptides. Viral proteins were subjected to electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide slab gels by the discontinuous buffer system of Laemmli (15). Sample preparation, electrophoresis, and staining were as described (25). Molecular weights were determined by the method of Weber and Osborne (26) with cytochrome *c* (11,700), chymotrypsinogen (27,500), ovalbumin (43,000), and bovine serum albumin (68,500) (Schwarz/Mann) as standards. Destained gels were photographed, and the negatives were scanned with a Photovolt photometer, model 520-A. Scans were recorded and peak areas were determined with a Hewlett-Packard 3380A integrator. Gels were dried with an SE-540 Hoefer Scientific Instruments slab gel dryer. For autoradiography, dried gels were placed in contact with Kodak X-Omat R film (XR-1). Fluorography of gels was conducted by the method of Bonner and Laskey (4). Films were developed in Kodak D-19, and scans were made as described above.

RESULTS

Purification and characterization of the protein matrix. It is well-documented that treatment of occluded baculoviruses with alkaline carbonate results in the solubilization of the protein matrix (2, 13, 16). This technique was used to isolate the matrix proteins from *P. interpunctella* GV. The solubilized matrix was separated from other viral components by centrifugation on glycerol gradients. It formed a

diffuse band which remained on top of the gradients (Fig. 1).

Polyacrylamide gel electrophoresis of the isolated matrix showed that it consisted of a mixture of polypeptides ranging in molecular weight from 10,000 to 28,000 (Fig. 2B). Most of these protein bands resulted from the activity of an alkaline protease associated with the GV (25). If the protease was inactivated before solubilization of the matrix, the proteins recovered on top of the gradient consisted primarily of a polypeptide having a molecular weight of 28,000 (Fig. 2A). Also observed in the matrix preparations were two to three minor polypeptides having approximate molecular weights of 66,000 to 74,000 (Fig. 2A and B).

Purification of enveloped nucleocapsids.

Carbonate treatment was also used to release the enveloped nucleocapsids from purified GV. After incubation in carbonate, the dissociated viral preparation was centrifuged on glycerol gradients. When [³H]thymidine-labeled virus was disrupted, a single peak of DNA-labeled virus was obtained in the gradients (Fig. 1). When ¹⁴C-amino acid-labeled GV was similarly treated, a portion of the protein label cosedimented with the DNA label while the majority of the radioactivity corresponding to the matrix proteins remained on top of the gradient. Elec-

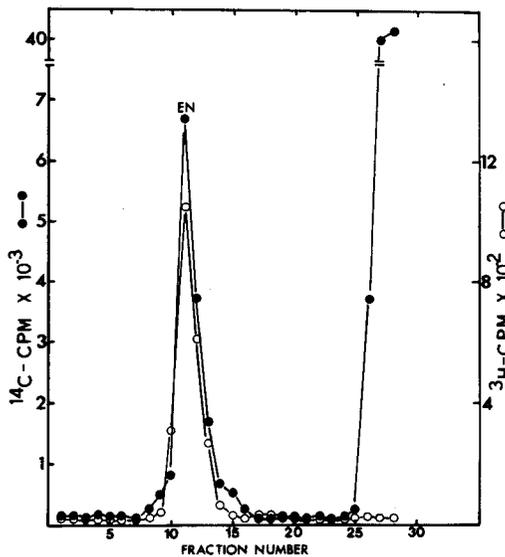


FIG. 1. Isolation of GV enveloped nucleocapsids (EN) by sedimentation in glycerol gradients. [³H]thymidine-labeled or ¹⁴C-amino acid-labeled GV was incubated in 0.05 M sodium carbonate-0.05 M NaCl, pH 10.6, for 15 min at room temperature. The dissociated GV was layered onto 30 to 70% glycerol gradients (in 0.01 M Tris-hydrochloride, pH 7.5) and centrifuged at 25,000 rpm (SW 41 rotor) for 1 h.

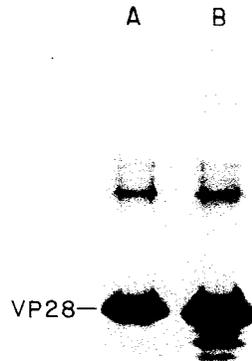


FIG. 2. SDS-polyacrylamide gel electrophoresis of GV matrix proteins. The protein matrix was solubilized and recovered on top of glycerol gradients as shown in Fig. 1. Samples were prepared for electrophoresis by boiling in 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol. (A) Matrix proteins isolated from GV heated before carbonate treatment for 30 min at 70°C to inactivate endogenous protease activity. (B) Matrix proteins isolated from protease-active GV.

tron microscopy of the band of virus obtained on these gradients showed that it consisted of a homogeneous preparation of enveloped nucleocapsids (Fig. 3A). Few degraded viral envelopes were observed, indicating that the carbonate treatment and sedimentation in glycerol gradients were not detrimental to the structural integrity of this viral component.

Purification of nucleocapsids. GV nucleocapsids were isolated by treating enveloped nucleocapsids with the nonionic detergent Nonidet P-40. Enveloped nucleocapsids, labeled *in vivo* with [³H]thymidine or ¹⁴C-amino acids, were dissociated with the detergent and then sedimented on 30 to 70% glycerol gradients. After centrifugation, essentially all of the DNA label was present in a single band which sedimented more slowly in the gradient than the enveloped nucleocapsids. Approximately 43% of the amino acid label cosedimented with the viral DNA label, whereas the remainder was localized near the top of the gradient (Fig. 4). Material from both regions of the gradient was recovered and examined for its polypeptide composition (results described below). The band of virus was recovered from the gradient and examined by electron microscopy to confirm that it contained nucleocapsids (Fig. 3B). The micrograph demonstrates that the nucleocapsids were structurally intact and free of contaminating envelope fragments.

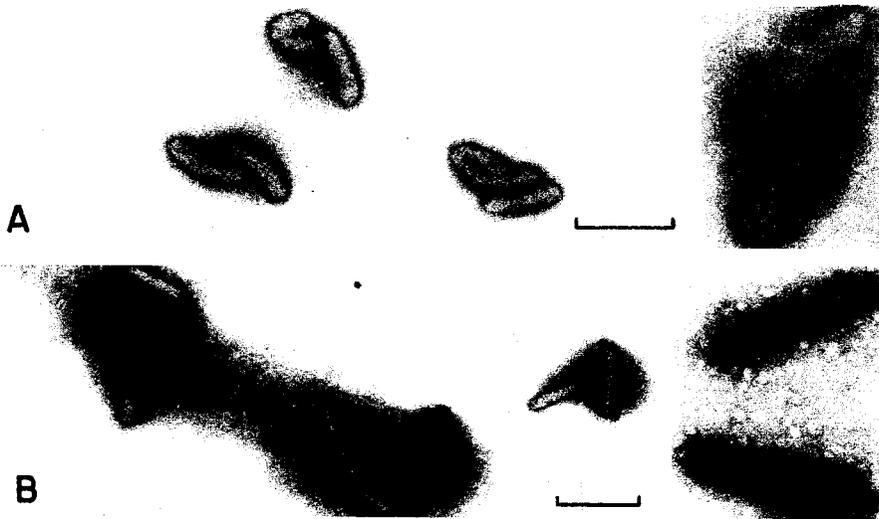


FIG. 3. Electron micrographs of purified GV enveloped nucleocapsids and nucleocapsids. Enveloped nucleocapsids (A) and nucleocapsids (B) were isolated as described in the legends to Fig. 2 and 4, respectively. Samples were mounted on Formvar-coated grids and stained with 1% phosphotungstic acid, pH 7.0. Bar represents 400 nm.

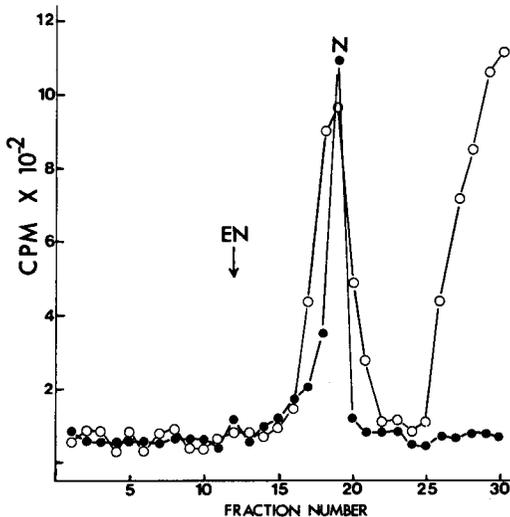


FIG. 4. Isolation of GV nucleocapsids (N) by sedimentation of Nonidet P-40-dissociated enveloped nucleocapsids in glycerol gradients. [^3H]thymidine-labeled (\bullet) or ^{14}C -amino acid-labeled (\circ) enveloped nucleocapsids were incubated in 1% Nonidet P-40 in 0.01 M Tris-hydrochloride, pH 8.5, for 30 min at room temperature. The preparation was layered on 30 to 70% glycerol gradients (in 0.01 M Tris-hydrochloride, pH 8.5) and centrifuged at 25,000 rpm (SW 41 rotor) for 1 h.

Identification of the structural proteins of enveloped nucleocapsids and nucleocapsids. Samples of purified enveloped nucleocapsids and nucleocapsids were dissociated with

SDS and mercaptoethanol. The proteins were subjected to electrophoresis in discontinuous SDS-polyacrylamide gels. An electropherogram of the structural polypeptides of the enveloped nucleocapsids is shown in Fig. 5 (EN). Fifteen proteins ranging in molecular weight from 12,600 to 97,300 were consistently identified. A variety of acrylamide concentrations (10, 12.5, 15 and 20%) was used to adequately separate proteins having similar electrophoretic mobilities. By using gradient gels ranging in acrylamide concentration from 10 to 26% or 7 to 15%, three to five additional minor viral protein species were resolved. The molecular weights of these minor structural proteins were 20,000, 36,000, 57,600, 59,800, and 86,000. The average molecular weight of each of the enveloped nucleocapsid proteins, determined by comparison with molecular weight standards, is presented in Table 1.

Eight polypeptides, ranging in molecular weight from 12,500 to 64,200, were observed when nucleocapsids were dissociated and electrophoresed in 12.5 and 15% SDS slab gels (Fig. 5, N). The average molecular weight of each nucleocapsid polypeptide is shown in Table 1. Two of the nucleocapsid proteins, VP49 and VP39, had molecular weights very similar to polypeptides identified as constituents of the viral envelope (results described below). They were, however, demonstrated to be unique nucleocapsid polypeptides by electrophoresis on 7 to 15% SDS-polyacrylamide gradient gels. This gel system provided for separation of enveloped nucleocapsid proteins VP48 and VP39 each into

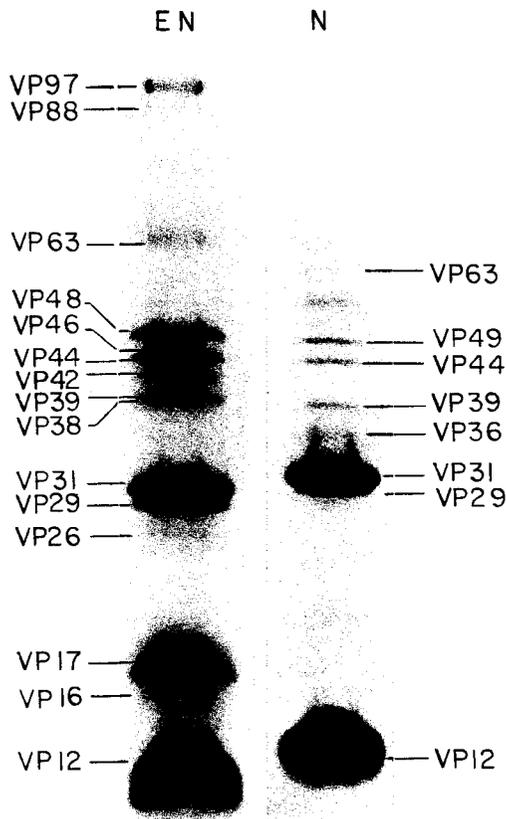


Fig. 5. SDS-polyacrylamide gel electrophoresis of GV enveloped nucleocapsids (EN) and nucleocapsids (N). Numerical designations refer to the molecular weight ($\times 10^{-3}$) of each polypeptide determined by comparison with molecular weight standards.

two distinct protein bands (results not shown). Only one of each of these polypeptides was present in nucleocapsids electrophoresed on the same gel. In addition to the eight polypeptides described above, minor protein components having approximate molecular weights of 27,000, 57,000, and 68,500 were observed in nucleocapsid preparations.

Protein composition of GV enveloped nucleocapsids and nucleocapsids. The relative amount of each viral polypeptide in enveloped nucleocapsids and nucleocapsids was estimated from densitometric scans of polyacrylamide gels in which unlabeled or ^{14}C -amino acid-labeled virus was resolved. The resulting distribution of protein among the various viral polypeptides is shown in Table 2. VP12, VP17, VP29, VP31, VP39, and VP48 were the predominant proteins in Coomassie-stained enveloped nucleocapsids, since they represented 40, 22, 20, 4, and 5% of the total protein in this viral component, respectively. The remaining polypeptides were present

TABLE 1. Molecular weights of GV enveloped nucleocapsid and nucleocapsid proteins

Enveloped nucleocapsids		Nucleocapsids	
Poly-peptide designation	Mol wt ^a	Poly-peptide designation	Mol wt ^b
VP97	97,300 \pm 3,300		
VP88	88,600 \pm 4,700		
VP63	63,700 \pm 2,500	VP63	64,200 \pm 3,000
		VP49 ^c	49,300 \pm 500
VP48	48,200 \pm 1,700		
VP46	46,800 \pm 1,500		
VP44 ^c	44,900 \pm 1,500	VP44 ^c	45,600 \pm 1,000
VP42	42,400 \pm 1,000		
VP39-E ^c	39,700 \pm 1,000		
		VP39-N	39,600 \pm 700
VP38	38,200 \pm 1,100		
		VP36	36,700 \pm 600
VP31	31,000 \pm 1,500	VP31	31,000 \pm 700
VP29 ^c	29,900 \pm 1,500	VP29 ^c	30,100 \pm 1,300
VP26	26,300 \pm 1,300		
VP17 ^c	17,800 \pm 600		
VP16	16,300 \pm 700		
VP12	12,600 \pm 500	VP12	12,500 \pm 300

^a Values reported are the means and standard deviations calculated from 10 separate determinations.

^b Values reported are the means and standard deviations calculated from eight separate determinations.

^c Phosphorylated polypeptides.

in amounts ranging from less than 1% (VP88 and VP38) to 2.8% (VP42). Scans of Coomassie-stained nucleocapsid proteins indicated that they consisted primarily of VP12 (54%) and VP29 and VP31 (41%). The other proteins associated with nucleocapsids were minor components which represented from 2.3% (VP39) to 3.1% (VP44) of the total nucleocapsid protein. A similar distribution of protein resulted from scans of fluorograms of gels containing ^{14}C -amino acid-labeled viral preparations except for VP12. Substantially more of this viral polypeptide appeared to be present in enveloped nucleocapsids and nucleocapsids, based on intensity of Coomassie staining, than on incorporation of ^{14}C -amino acids (Table 2). The lower percent values obtained by incorporation of amino acids most likely can be accounted for by the extremely high arginine content of VP12 (manuscript submitted). Polypeptides of high arginine content stain more intensely with Coomassie brilliant blue than do those with lower arginine content (D. J. Stubs, M.S. thesis, Kansas State University, Manhattan, Kansas, 1978).

Identification of viral envelope proteins. Radioiodination is a useful technique for examining the distribution and organization of proteins in animal viruses (19, 27). A gentle *in vitro*

TABLE 2. Protein composition of GV enveloped nucleocapsids and nucleocapsids

Enveloped nucleocapsids			Nucleocapsids			
Viral polypeptide	Total protein (%)		Viral polypeptide	Total protein (%)		
	Stained ^a	Incorporation ^b		Stained ^a	Incorporation ^b	
VP97	2.7	5.9				
VP89	— ^c	— ^c				
VP63	1.0	1.4	VP63	2.6	2.8	
			VP49	2.5	3.3	
VP48	5.0	8.9				
VP46	1.8	9.4	VP44	3.1	3.1	
VP44	1.2					
VP42	2.8					
VP39-E	4.1	5.7				
VP38	— ^c	— ^c	VP39-N	2.3	2.2	
			VP36	— ^c	— ^c	
VP31, VP29	20.7	19.1	VP31, VP29	40.9	39.2	
VP26	— ^c	— ^c				
VP17	22.3	22.9				
VP16	— ^c	— ^c				
VP12	40.8	27.3	VP12	54.2	44.5	

^a Percentage of total protein determined by calculating the area under each Coomassie brilliant blue-stained peak; values represent the average of six separate determinations.

^b Percentage of total protein determined from area under each peak resulting from autoradiography of SDS gels of virus labeled in vivo with ¹⁴C-amino acids; values reported are the means of three separate determinations.

^c Peak areas less than 1% of the total protein.

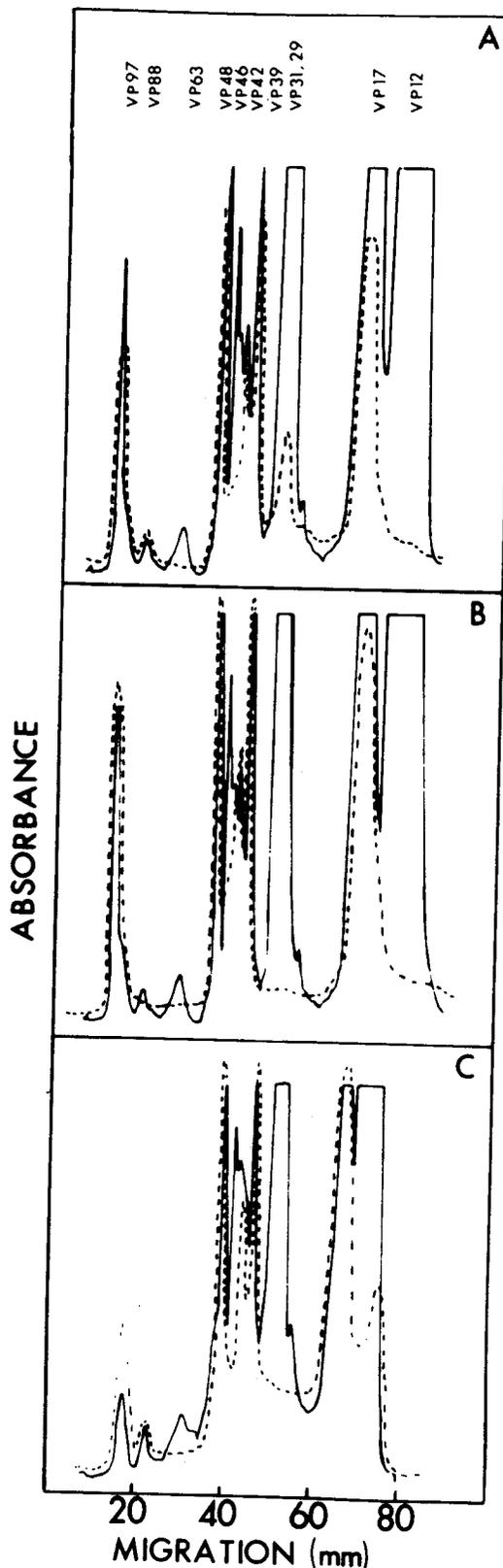
labeling procedure employing solid-phase bound lactoperoxidase (Enzymobead reagent, Bio-Rad) and [¹²⁵I]iodine was used to identify GV envelope proteins. The large size of the bound enzyme inhibits its penetration into the viral membrane so that only those proteins external to the viral envelope are labeled (18, 27).

To verify the specificity of the labeling procedure used, iodinated enveloped nucleocapsids were incubated with the proteolytic enzyme trypsin (2 mg/ml) for 2 h at 37°C so that proteins located on the outer surface of the viral envelope would be digested (18, 20). After trypsin treatment, the enveloped nucleocapsids were reisolated by centrifugation in glycerol gradients. It was found that the majority of the iodine label was removed from the virus by the protease and remained on top of the gradient. Electron microscopy of the band of virus obtained in these gradients revealed that it consisted of particles with intact envelopes (results not shown) indicating that the trypsin did not alter the integrity of the envelopes. It was concluded from these data that the lactoperoxidase labeling was specific for proteins external to the viral envelope.

To identify which viral proteins were labeled by the lactoperoxidase procedure, and thus were envelope constituents, portions of the iodinated enveloped nucleocapsids were dissociated by SDS and mercaptoethanol and the proteins were

resolved by electrophoresis in 12.5% discontinuous SDS gels. Densitometer tracings of an autoradiogram of such a gel (Fig. 6A) showed that the iodine label was localized at positions corresponding to VP17, VP39, VP42, VP48, and VP97. Of these polypeptides, VP17 was the major component. The small amount of label associated with nucleocapsid proteins VP29 and VP31 probably resulted from some envelope disruption during preparation of enveloped nucleocapsids for iodination.

NP-40 treatment of the iodinated enveloped nucleocapsids provided further evidence that these five structural polypeptides were envelope proteins. Viral proteins that were solubilized by NP-40 were precipitated with 25% trichloroacetic acid and resolved in polyacrylamide gels. Autoradiography revealed that the label migrated with electrophoretic mobilities characteristic of VP17, VP39, VP42, VP48, and VP97 (Fig. 6B). The viral proteins which were released by NP-40 were also examined after their electrophoresis in SDS discontinuous gels and staining with Coomassie brilliant blue. As shown in Fig. 6C, the proteins that were visualized again were VP17, VP39, VP42, VP48, and VP97. A small amount of VP12 was also observed in these gels. Its presence may indicate that some nucleocapsid disruption occurred during detergent treatment.



Modifications of GV structural polypeptides. (i) Glycosylation. A sensitive technique for detection of carbohydrate moieties on polypeptides consists of radiolabeling with [³H]sodium borohydride after neuraminidase and galactose oxidase treatment (3, 9). This procedure was used to determine whether any of the structural polypeptides of *P. interpunctella* GV were glycosylated. Even after prolonged exposure (4 months) of gels containing radiolabeled virus to X-ray film, no radioactivity was detected in any of the polypeptides associated with granulin, enveloped nucleocapsids, or nucleocapsids. Under the same conditions of labeling and exposure, the two structural glycoproteins of Sendai virus were extensively labeled. A labeling procedure specific for sialic acid residues (3) also failed to reveal any glycosylation in GV.

Glycosylation of viral polypeptides was also investigated by SDS gel electrophoresis and fluorography of viral components isolated from infected larvae that were injected with [¹⁴C]fucose (15 Ci/mmol, New England Nuclear) or [¹⁴C]glucosamine (30 Ci/mmol, New England Nuclear). Again, no incorporation of isotope by any of the GV structural proteins was observed.

(ii) Phosphorylation. Preliminary experiments were conducted to determine whether GV structural polypeptides were phosphorylated. ³²P-labeled virus was produced by injecting 1 μl (25 mCi/ml) of [³²P]orthophosphoric acid (neutralized to pH 7.4 with 1 M Tris-hydrochloride) into GV-infected larvae. After purification of the virus from the larvae, the granulin, enveloped nucleocapsids, and nucleocapsids were isolated, dissociated with SDS and mercaptoethanol, and electrophoresed on SDS-polyacrylamide gels. The association of radioactive phosphate with viral polypeptides was determined by compari-

FIG. 6. Identification of GV envelope proteins. (A) Surface-iodinated enveloped nucleocapsids were dissociated with SDS and mercaptoethanol, and the proteins were resolved by SDS gel electrophoresis. Viral polypeptides were identified by comparing densitometer tracings of autoradiograms of the gels (----) to those of Coomassie blue-stained gels (—). (B and C) Iodinated or unlabeled enveloped nucleocapsids were incubated in 1% Nonidet P-40 for 30 min at room temperature. The solubilized proteins were isolated as shown in Fig. 4 and precipitated with 25% trichloroacetic acid. The precipitates were washed with ethanol, dissociated with SDS and mercaptoethanol, and resolved by SDS gel electrophoresis. Envelope proteins were identified by comparing densitometer tracings of autoradiograms (B) or of stained gels (C) containing the solubilized proteins (----) to those of stained gels containing enveloped nucleocapsids (—).

son of densitometer tracings of autoradiograms of each gel to those of the same gel stained with Coomassie brilliant blue. The major constituent of the protein matrix, granulin, was observed to be phosphorylated (Fig. 7A). Of the proteins present in enveloped nucleocapsids, VP17, VP29, VP39, VP44, and VP48 contained significant amounts of ^{32}P -label (Fig. 7B). This resulted from incorporation of the isotope by two of the envelope proteins, VP17 and VP39. The remaining three phosphorylated species, VP29, VP44, and VP49, were associated with nucleocapsid preparations (Fig. 7C).

DISCUSSION

The techniques described in this paper provided for the separation of the GV of *P. interpunctella* into protein matrix, enveloped nucleocapsid, nucleocapsid, and solubilized envelope components. By isolation of each viral component and analysis of its polypeptide composition, the number and organization of the major proteins in the structurally complex GV could be elucidated. The predominant constituent of the protein matrix had a molecular weight of 28,000. In this property, it resembled the matrix proteins that have been characterized in other baculoviruses (6, 11, 16, 22). The high-molecular-weight proteins associated with the granulin appear to be unique to the protein matrix, since their electrophoretic mobilities were different from that of the polypeptides found in either the enveloped nucleocapsids or nucleocapsids. McCarthy and Liu (16) have also observed a high-molecular-weight protein in the protein matrix prepared from the NPV of *Porthetria dispar*. The distribution of these minor proteins within the protein matrix, as well as their biochemical properties and importance to the viral infection process, remains to be determined.

The number of polypeptides observed in the enveloped nucleocapsids and nucleocapsids was dependent on the resolving power of the SDS gel in which they were electrophoresed. Generally, 15 polypeptides were present in enveloped nucleocapsids and 8 were found in nucleocap-

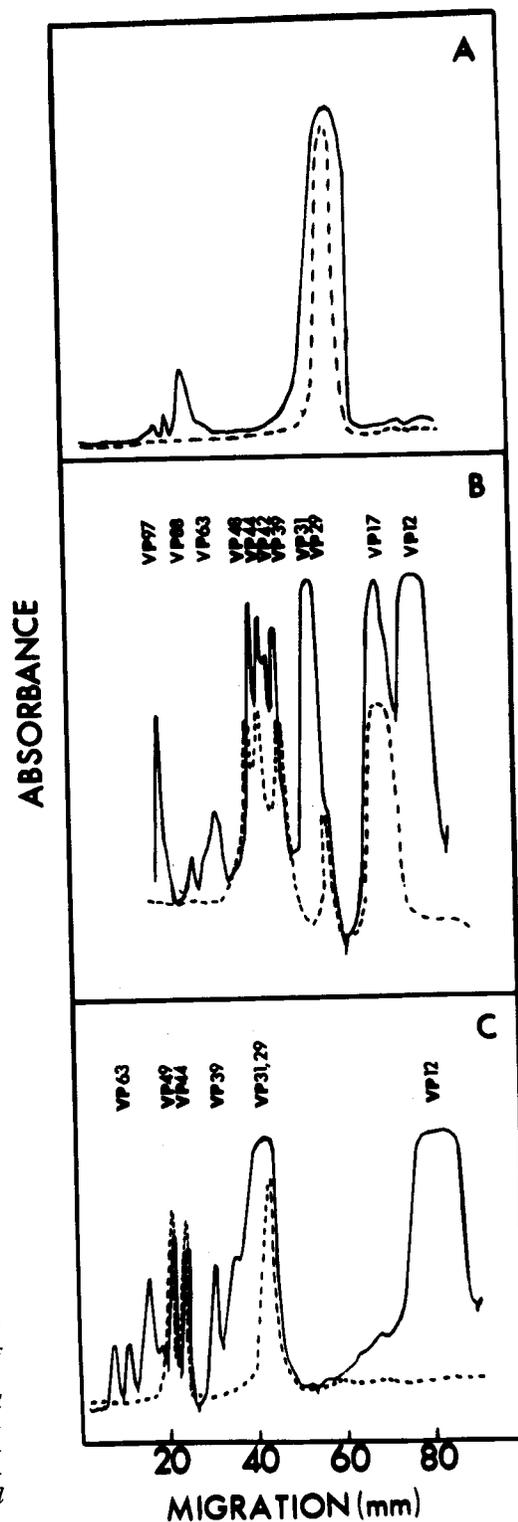


FIG. 7. Identification of phosphorylated GV structural polypeptides. Protein matrix (A), enveloped nucleocapsids (B), and nucleocapsids (C) were isolated as described from GV grown in the presence of [^{32}P]orthophosphate. Each viral component was solubilized by boiling in SDS and mercaptoethanol, and its polypeptides were resolved by SDS gel electrophoresis. Phosphorylated viral polypeptides were identified by comparing densitometer tracings of autoradiograms of each gel (-----) to those of the same gel stained with Coomassie brilliant blue (—).

sids. The minor protein species associated with these viral components may represent cleavage products or modified (phosphorylated or acetylated) forms of the major viral proteins. Two-dimensional gel electrophoresis is currently being used to investigate these possibilities as well as to separate the polypeptides of enveloped nucleocapsids and nucleocapsids into more clearly distinguishable proteins.

Iodination studies showed that at least five of the proteins found in enveloped nucleocapsids were located on the outer surface of the viral envelope. Previous studies comparing the polypeptide compositions of GV and NPV enveloped nucleocapsids and nucleocapsids have implicated certain proteins as possible viral envelope components (5, 7, 11). The lactoperoxidase labeling procedure used in this work provided a direct means of identifying these proteins as VP17, VP39, VP42, VP48, and VP97. Nonidet P-40 treatment of the enveloped nucleocapsids provided further evidence for localization of these proteins in the viral envelope. These polypeptides were readily released from the viral structure during solubilization of the envelope by the detergent.

There were a few additional viral polypeptides (VP38, VP16, and VP46) which were present in enveloped nucleocapsids but not in nucleocapsids. Although this observation suggests that these proteins are envelope proteins, they did not appear to be labeled by the radioiodination procedure. It is possible that these proteins are located more internally in the viral membrane than the other envelope proteins, and, as a result, are unexposed to the lactoperoxidase. They may also be components of an intermediate layer which has been proposed by Kawamoto et al. from electron microscopic observations (12) to exist between the envelopes and nucleocapsids. This intermediate layer may be analogous to the "membrane" (M) proteins associated with the envelopes of rhabdoviruses, orthomyxoviruses, and paramyxoviruses which are speculated to serve as recognition sites for assembly of the nucleocapsids into envelopes (17).

The number and relative amounts of structural polypeptides observed in the GV based on in vivo incorporation of ^{14}C -amino acids into viral proteins were very similar to those based on intensity of Coomassie brilliant blue staining. These results indicate that preexisting host proteins are not incorporated to any great extent into GV during its replication and suggest that the structural proteins are virus specified. The possibility is not ruled out, however, that some of the proteins observed in the GV are host-contributed polypeptides that are synthesized

after initiation of virus infection. Because the effect of GV infection on host protein synthesis has not been determined, the specific association of cellular proteins with the structural components of GV cannot be further evaluated at present.

None of the GV structural polypeptides was radiolabeled by in vitro labeling procedures specific for carbohydrate residues on proteins. This may indicate that: (i) none of the viral polypeptides contains carbohydrate moieties, (ii) some of the structural polypeptides are glycosylated but with residues other than the sugars sialic acid, galactose or *N*-acetyl galactosamine which the in vitro labeling procedure are specific for, or (iii) some of the polypeptides are glycoproteins containing galactose or *N*-acetyl galactosamine residues that are so highly substituted by other sugars such as fucose that they do not become labeled. It is difficult, at present, to rule out any of these possibilities, particularly because viral polypeptides synthesized in larvae in the presence of [^{14}C]fucose or [^{14}C]glucosamine may not have incorporated label to a detectable specific activity. These results are especially surprising in the case of the envelope proteins. All other well-characterized proteins found on the outer surface of animal cell membranes or animal virus envelopes have been found to be glycoproteins (9).

The 80×10^6 -dalton genome of the GV of *P. interpunctella* (24) is genetically complex enough to code for approximately 150 proteins having an average molecular weight of 42,000. To synthesize the total number of structural polypeptides identified so far in this virus, it would be necessary to utilize only 20 to 25% of the DNA molecule. This indicates that the GV genome probably also contains information to code for nonstructural proteins having regulatory or enzymatic activities. Whether virus-coded enzymes or regulatory proteins such as early proteins are involved in the viral replication process has not been investigated.

Comparison of the polypeptide composition of the GV of *P. interpunctella* to that of the GVs of *T. ni*, *S. frugiperda*, and *P. brassicae* reveals several differences, based on electrophoretic mobilities, in the structural proteins of these viruses. These differences appear to be more profound for the proteins of the enveloped nucleocapsids than for those of the nucleocapsids, indicating that a major divergence between these GVs may be due to their envelope proteins. If the envelope proteins are responsible for the tissue and host specificity exhibited by the GV, changes in their composition may account for intrinsic differences observed in the biological

activity of these viruses. From these observations, the importance of determining the number and structural position of the viral polypeptides for elucidating their biological function becomes obvious. This information is also necessary for the development of immunological reagents precise enough to provide satisfactory identification of baculoviruses as well as to analyze specific steps in the infection process of these complex viruses.

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