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Occluded and Budded *Autographa californica* Nuclear Polyhedrosis Virus: Immunological Relatedness of Structural Proteins

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The immunological relatedness of the structural proteins of the budded and occluded phenotypes of *Autographa californica* nuclear polyhedrosis virus was examined by reciprocal immunoblotting and by in situ peroxidase-antiperoxidase staining of virus-induced cell surface and intracellular antigens with antisera to both phenotypes. The molecular weights (MWs) of major structural proteins of both phenotypes that reciprocally cross-reacted were 92,500, 78,000, 62,500, 54,000, and 42,000. A highly immunogenic, major structural protein of the occluded phenotype of 46,000 MW was not recognized by antiserum to the budded phenotype, and a major structural protein of the budded phenotype, 48,000 MW, was not recognized by antiserum to the occluded phenotype. Both the budded and occluded phenotypes contained a protein of 33,500 MW that comigrated with polyhedrin (the matrix protein) and reacted with antiserum and monoclonal antibody to polyhedrin. Evidence was obtained for the apparent antigenic relatedness of proteins of different MWs from the budded and occluded phenotypes, possibly indicative of different processing of some proteins for the two phenotypes. Antiserum to the occluded phenotype recognized virus-induced cell surface antigens, indicating antigenic similarities between the occluded phenotype and envelope proteins of the budded phenotype. Antiserum to the budded phenotype recognized viral proteins produced before the appearance of cytopathic effect, whereas antiserum to the occluded phenotype did not.

One unusual and intriguing feature of the life cycle of *Autographa californica* nuclear polyhedrosis virus (AcNPV), a member of the family Baculoviridae, is that two distinct phenotypes—the plasma membrane-budded form (BV) and the occluded form—are produced and appear to have separate roles in the persistence of the virus in nature (1). The occluded virus can be released from its surrounding protein matrix by exposure to alkali. It is this *l*arvae-*o*ccluded *v*irus, *a*lkali *l*iberated (LOVAL), that has been compared with BV and found to be different in several respects. BV and LOVAL are morphologically different in that the budded forms occur primarily as single nucleocapsids per envelope; the envelopes, which the nucleocapsids gain as they bud through the plasma membrane, are large and loose fitting, and surface projections are apparent (1, 7, 8, 16, 19). The LOVAL forms, on the other hand, are mostly multiple nucleocapsids contained within tight-fitting envelopes that are newly synthesized in the nucleus and appear to be without peplomers. There are differences in their infectivity both in vivo and in vitro (23) and in their ability to be neutralized by homologous and heterologous antisera (24). There are molecular weight (MW) differences in some of the structural proteins of the two phenotypes (5, 15, 19), and some proteins of the same MW have been found to be quantitatively different or differently phosphorylated (12, 15). Only one report has been published comparing the antigenic relatedness of the structural proteins of the two AcNPV phenotypes, and in that study antiserum to only one phenotype was used (14). In the studies presented here, the structural proteins of the two phenotypes were compared by reciprocal immunoblotting (Western blotting) and in situ peroxidase-antiperoxidase (PAP) staining of virus-induced cell surface and intracellular antigens with antisera to both phenotypes. Among the findings were that antiserum to LOVAL recognized virus-induced cell surface antigens, indicating there were antigenic similarities between LOVAL and the envelope proteins of BV. Antiserum to BV recognized viral proteins produced earlier in infection than did antiserum to LOVAL. The MWs of major structural proteins of LOVAL and BV that reciprocally cross-

reacted were 92,500, 78,000, 62,500, 54,000, and 42,000. A highly immunogenic, major structural protein of LOVAL of 46,000 MW was not recognized by antiserum to BV, and a major BV structural protein of 48,000 MW was not recognized by antiserum to LOVAL. Both BV and LOVAL contained a protein of 33,500 MW that comigrated with polyhedrin and reacted with antiserum and monoclonal antibody to polyhedrin. Evidence was obtained for the apparent antigenic relatedness of LOVAL and BV proteins of different MWs, possibly indicative of different processing of some proteins for the two phenotypes.

MATERIALS AND METHODS

Virus. The virus used was the cloned E2 variant of AcNPV (13) and was supplied originally by M. D. Summers, Texas A & M University, as cell culture-derived polyhedra. These polyhedra were used to infect early fourth-instar *Trichoplusia ni* larvae per os. Hemolymph from the infected *T. ni* larvae was used to infect IPLB-SF-21 cells to generate plasma membrane-budded virus (BV). BV used in this study was the third-passage virus from the 48-h culture medium of infected IPLB-SF-21 cells and was purified by differential centrifugation and sucrose density gradients as described previously (24). Polyhedra from infected *T. ni* larvae were purified as follows. Diseased larvae were extracted in 0.01 M Tris buffer (pH 7.8)–0.001 M EDTA–0.1% 2-mercaptoethanol (ca. 1 g of larvae per 2 ml of buffer) in an Acme juicer (model 6001). The liquid extract was incubated overnight at 4°C. General floating debris was removed and discarded the next morning. The remaining extract was diluted 1:6 with 0.01 M Tris buffer (pH 7.8)–0.001 M EDTA (Tris-EDTA buffer) and layered in 5-ml portions on 30-ml, 40 to 63% sucrose gradients. The gradients were centrifuged for 30 min at 82,000 × *g* (average). The bands of polyhedra were removed, diluted with distilled water, and pelleted by centrifugation at 12,000 × *g* for 30 min. The pelleted polyhedra were either stored at −70°C or suspended in distilled water. The resuspended polyhedra were solubilized in dilute alkaline saline (0.1 M Na_2CO_3, 0.15 M NaCl, pH 10.9) at 5 mg/ml for 15 min at room temperature before being centrifuged on 25 to 59% sucrose gradients for 45 min at 4°C and 82,000 × *g* (average) as above. The bands of virions were removed from the gradients, diluted 1:3 in Tris-EDTA buffer, and pelleted by centrifugation at 22,000 × *g* for 45 min.

Cell culture. Cell lines IPLB-SF-21 (*Spodoptera frugiperda*) and TN-368 (*T. ni*) were used in this study. BV used in antiserum production and in biochemical studies was produced in IPLB-SF-21 cells grown at 28°C in TC-100 medium (6) with 10% rabbit serum in 150-ml Corning flasks. For surface and intracellular antigen studies, the medium contained 10% fetal calf serum instead of rabbit serum. TN-368 cells were grown at 28°C in TNM-FH medium with 10% fetal calf serum (9).

AcNPV polyhedrin preparation. Gradient-purified *T. ni*-derived polyhedra suspended at 5 mg/ml in Tris-EDTA buffer were heated for 2 h at 70°C and then pelleted by centrifugation at 12,000 × *g* for 30 min. The polyhedra were rinsed twice by two cycles of suspension in distilled water and repelleting. The polyhedra were then suspended in 0.01 M $HgCl_2$–0.01 M Tris buffer (pH 7.8) and incubated overnight at 4°C. The next morning they were pelleted and rinsed in distilled water five times before being dissolved by overnight incubation at 4°C in dilute alkaline saline. The preparations were cleared of virions, polyhedral remnants, and other debris by centrifugation at 100,000 × *g* for 30 min, and the supernatant fluid containing the polyhedrin was collected and stored in portions at −70°C. The purity and integrity of the polyhedrin protein preparation was checked by polyacrylamide gel electrophoresis (PAGE) before it was used as antigen for antiserum production. Protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.).

Antisera production. Antisera to BV, LOVAL, and polyhedrin were made in New Zealand white rabbits. All rabbits were bled before receiving their first injection of antigen. This injection consisted of 80 μg of antigen emulsified 1:1 with complete Freund adjuvant and delivered subcutaneously to two sites along the back. The second and all subsequent injections consisted of 80 μg of antigen emulsified 1:1 in incomplete Freund adjuvant. The second injection was delivered 1 week after the first, subcutaneously; and the third, fourth, and fifth samples were each given intramuscularly at 2-week intervals thereafter. Blood samples were taken from the rabbits after the last three injections. When tests conducted by indirect enzyme-linked immunosorbent assay (ELISA) (17) indicated that booster injections no longer increased the titer significantly, the rabbits were bled out by cardiac puncture. All sera were stored at −70°C. BV antiserum used in these studies was adsorbed before use with about 6×10^6 IPLB-SF-21 cells per ml of serum.

Monoclonal antibody. Monoclonal antibody to polyhedrin was prepared and kindly provided by Yuan-Shen Huang and Clinton Kawanishi of the Environmental Protection Agency, Research Triangle Park, N.C. The antibody was produced to the polyhedrin of *Heliothis zea* nuclear polyhedrosis virus, but cross-reacted with AcNPV polyhedrin. The antibody used in this study was made by the clone 1003-2-2.

PAGE and electroblotting. Viral proteins were analyzed by sodium dodecyl sulfate (SDS)-PAGE on 10 or 12.5% (0.75- or 1.5-mm) slab gels as described by Laemmli (10). Electrophoresis was at 30 mA for 3 h. Viral proteins were stained in the 0.75-mm gels with silver stain (Bio-Rad Laboratories). MW standards (Bio-Rad Laboratories) and their MWs were as follows: lysozyme, 14,400; soy bean trypsin inhibitor, 21,500; carbonic anhydrase, 31,000; ovalbumin, 45,000; bovine serum albumin, 66,200; and phosphorylase B, 92,500.

Immunological staining of blotted proteins. The procedure used for staining blotted proteins was similar to the indirect peroxidase method described by Towbin et al. (17), with several details changed.

Nitrocellulose sheets containing the electrophoretically transferred viral proteins were placed face up in smooth microtiter plate lids and soaked for 30 min at 37°C in Clint blocking buffer (CBB; 0.05 M Tris buffer [pH 7.5], 0.15 M NaCl, 0.005 M EDTA, 0.25% gelatin, 0.05% Nonidet P-40, and 2% calf serum) to saturate unoccupied protein binding sites. After removal of the

CBB, antiserum (or monoclonal antibody) diluted 1:20 in CBB was added, and the tray was placed on a rocker platform in a 37°C incubator for 2 h. The angle and frequency of oscillation was adjusted so that the antiserum constantly coated the surface of the blot. A 4-ml volume of diluted antiserum was usually sufficient. The diluted antiserum (antibody) was used one time only. After this 2-h incubation, the blot was rinsed for 5 min with a generous amount of phosphate-buffered saline (pH 7.4) containing 0.05% Nonidet P-40, followed by two 5-min rinses in CBB. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) (or rabbit anti-mouse IgG) (Sigma Chemical Co., St. Louis, Mo.), diluted 1:100 in CBB, was then added, and the blot was incubated for 1 h as before. After this incubation the blot was rinsed once with phosphate-buffered saline containing 0.05% Nonidet P-40, as before, and twice with phosphate-buffered saline alone. After the last rinse, the blots were stained with a solution of 0.05% diaminobenzidene tetrahydrochloride in 0.05 M Tris buffer (pH 7.5)–0.01% H_2O_2, usually for 1 to 5 min. The blots were rinsed in water and dried.

Preparation of uninfected cell extract. Log- and stationary-phase IPLB-SF-21 cells were mixed and pelleted by centrifugation at 1,000 × *g* for 5 min. The cells were suspended in phosphate-buffered saline (ca. 1 ml per 0.5 ml of packed cells) and aspirated about 20 times through a 23-gauge needle attached to a 3-ml syringe. Large cellular debris was pelleted by centrifugation for 5 min at 1,000 × *g*. The supernatant fraction was stored in 0.1-ml portions at −70°C for later use as the uninfected cell extract control.

PAP assay for virus-induced surface and intracellular antigens. PAP assays were performed by the method described previously, except the cells were fixed for 20 min in 4% phosphate-buffered (0.1 M, pH 7.1) glutaraldehyde or for 30 s in buffered Formol acetone (pH 4.5) as noted (20, 21).

RESULTS

Analysis of LOVAL by SDS-PAGE and reciprocal blots. Altogether, at least 35 different LOVAL proteins reacted with one or both antisera. The LOVAL blots incubated with homologous antiserum were not noticeably stained more intensely overall than were the blots incubated with anti-BV serum (Fig. 1). There were considerable staining differences, however. Major reacting proteins of MWs 92,500, 78,000, 62,500, 54,000, and 42,500 were evident when blots were incubated with both heterologous and homologous antisera, but two additional proteins of 46,000 and 36,000 MW showed major staining reactions only with the homologous antiserum. There was no evidence of recognition of the 46,000-MW protein by antiserum to BV, which was the most highly stained LOVAL protein with anti-LOVAL serum. Relatively minor proteins that were not recognized by anti-BV serum but were detected in blots of high LOVAL protein concentration by anti-LOVAL serum were the 150,000- and 29,000-MW proteins (Fig. 1). The 36,000-MW protein reacted more strongly with anti-LOVAL serum than did the 34,000-MW protein, which appeared on the gel to be in greater abundance. Both of these proteins were only faintly recognized by anti-BV serum, if at all. In contrast, LOVAL protein of 48,000 MW apparently was not recognized by antiserum to LOVAL, but was recognized by anti-BV serum. Some other proteins of MW 71,000, 52,000, 39,500, 32,000, and 14,000 stained more intensely with anti-BV serum than with anti-LOVAL serum. Curiously, no BV protein in the 32,000-MW region stained as intensely with anti-BV serum as the LOVAL 32,000-MW protein did (Fig. 1, 2, and 3). The 45,000-MW protein of LOVAL, a major protein as revealed by the silver-stained gel, did not stain with antiserum to LOVAL.

Analysis of BV by SDS-PAGE and reciprocal blots. Careful inspection of the BV blots (Fig. 2 and 3) revealed that at least 31 BV proteins reacted with antiserum to BV or LOVAL or both. Sixteen proteins reacted with both antisera, although staining intensity on the whole was greater with anti-BV serum.

The reaction patterns produced by staining BV blots incubated with anti-BV serum closely resembled the silver-stained gel of BV, but there were some notable differences. Proteins of MW

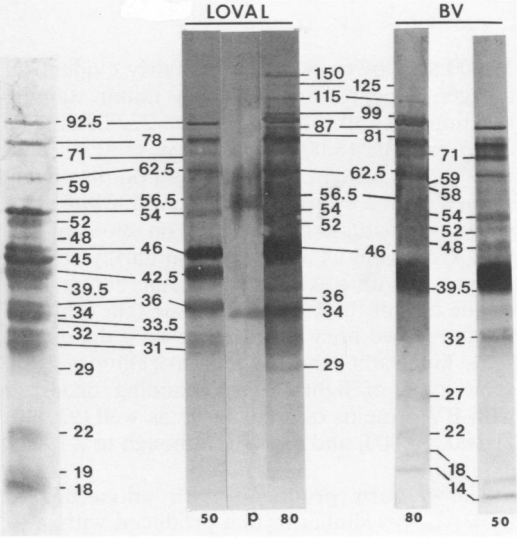

FIG. 1. SDS-PAGE of LOVAL and immunoblots of LOVAL and polyhedrin (P). Silver-stained LOVAL proteins after SDS-PAGE on 12.5% gels are compared with LOVAL and polyhedrin immunoblots from comparable gels. The concentration of virus (micrograms) is indicated at the bottom of the blots, and the antiserum is indicated at the top. About 40 μg of viral protein was used in the silver-stained gel. MWs (in thousands) of the proteins are indicated.

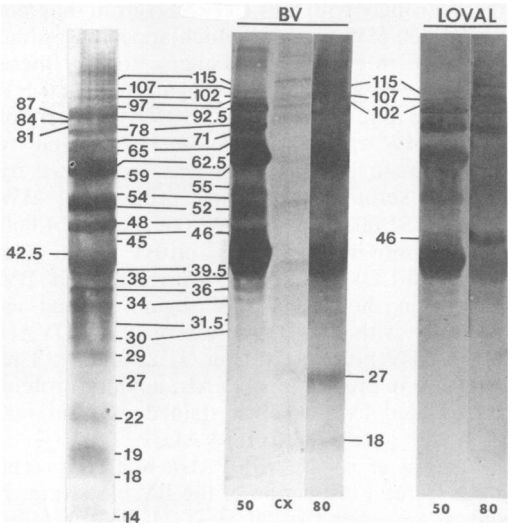

FIG. 2. SDS-PAGE of BV and immunoblots of BV and uninfected cell extract (CX). Silver-stained BV proteins after SDS-PAGE on 12.5% gels are compared with BV and cell extract immunoblots from comparable gels. The concentration of virus (micrograms) is indicated at the bottom of the blots, and the antiserum is indicated at the top. About 40 μg of viral protein was used in the silver-stained gel, and about 100 μg of cell extract was used in the immunoblot. MWs (in thousands) of proteins are indicated.

36,000 and lower, which were fairly evident on the gel, appeared to elicit only minor staining reactions. An exception was the 27,000 (Fig. 2 and 3) and the 18,000 (Fig. 3) MW proteins. The 48,000-MW protein, a major band on the silver-stained gel, appeared as a layered white substance on a dark background on blots of the 12.5% gels and as a broadly and darkly stained perimeter with a whitish overlying center area on the blot of the 10% gel (Fig. 3). The white of the unreacted area appeared to be a deposit of some kind rather than blank nitrocellulose. This same type of light-on-dark banding occurred with BV proteins of other MWs as well (92,500, 71,000, 54,000, and 52,000), although to a lesser extent.

The pattern produced with antiserum to LOVAL was similar to that produced with antiserum to BV, except for the complete absence of staining with the former of the 48,000-MW protein (Fig. 2 and 3) and the absence of, or only very faint, staining for the 71,000- and 52,000-MW proteins and for proteins of MW 38,000 or lower, including the 36,000- and 34,000-MW proteins (Fig. 2). Antiserum to LOVAL also reacted with 46,000- and 65,000-MW proteins, whose possible reactions to anti-BV serum were obscured by the neighboring massive reactions of the 42,500- and the 62,500-MW proteins, respectively (Fig. 3). Curiously, at least two BV proteins that appeared not to have LOVAL counterparts of the same MWs, 102,000 and 65,000, reacted with antiserum to LOVAL (Fig. 3). For both BV and LOVAL, some proteins visible in the silver-stained gels did not react on the blots, and other proteins inapparent in the gels were stained on the blots (Table 1).

Reaction of LOVAL and BV blots with anti-polyhedrin antibodies. Comparison of LOVAL and BV blots incubated with anti-polyhedrin serum revealed that a 33,500-MW protein present in both comigrated with the polyhedrin monomer and reacted with the antiserum (Fig. 4A). BV proteins of MWs 54,000 and 29,000 were also faintly stained. It was noted that in addition to the band of the 33,500-MW polyhedrin monomer, the polyhedrin blot showed staining of proteins of 64,000 and 59,000 MW and several of MWs below 33,500. A control blot incubated with normal rabbit serum showed no reaction (Fig. 4C).

That these reactions were specifically attributable to anti-polyhedrin antibodies in the antiserum was confirmed by incubating blots of LOVAL, BV, and polyhedrin with a monoclonal antibody to polyhedrin. The results (Fig. 4B) showed that the 33,500-MW proteins in all three

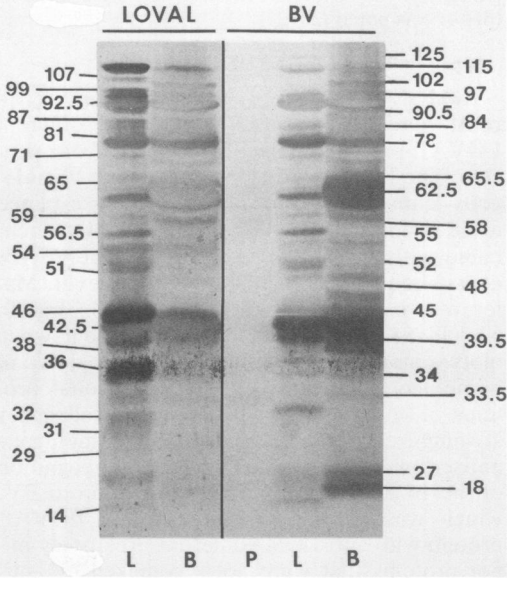

FIG. 3. Immunoblots of BV (B), LOVAL (L), and polyhedrin (P) from 10% SDS-polyacrylamide gels. The antiserum used is designated at the top. MWs (in thousands) are indicated.

TABLE 1. AcNPV LOVAL and BV structural proteins detected by silver or indirect PAP staining

MW of protein (10^3)	Protein stained by:[a]		MW of protein (10^3)	Protein stained by:[a]	
	BV	LOVAL		BV	LOVAL
150		l	52	l* b s	l b s
125		l b	51		l b
115	l b s	l b	48	b s	b s
107	l b s	l	46	l s	l s
102	l b s		45	b s	s
99		l b	42.5	l b s	l b s
97	l b s	l b	39.5	l b s	l b s
92.5	l b s	l b s	38	l* b s	l b
90.5	b		36	l* b s	l b* s
87	b s	l	34	l* b s	l b* s
84	l b s	l b	33.5	b	l s
81	l b s	l b	32		l b s
78	l b s	l b s	31.5	b s	
71	l b s	l b s	31		l b s
65.5		l b	30	b s	
65	l s		29	s	l s
62.5	l b s	l b s	27	b s	l b
59	l b s	l b s	22	s	b s
58	b	l b	19	s	s
56.5		l b s	18	b s	b s
55	b		14	s	l b
54	l b s	l b s			

[a] AcNPV LOVAL and BV structural proteins were subjected to SDS-PAGE and either stained in the gel by the silver staining method (s) or transferred to nitrocellulose and stained by indirect PAP with antiserum to BV (b) or to LOVAL (l). Reactions shown with an asterisk (*) were equivocal.

blots reacted with the antibody, although not as intensely as they did with the antiserum. The BV protein of MW 29,000 did not stain above the background, but the 64,000- and 59,000-MW proteins on the polyhedrin blot did.

PAP staining of virus-induced cell surface and intracellular antigens. Foci of infected TN-368 and IPLB-SF-21 cells were fixed with either Formol buffered acetone or 4% glutaraldehyde and stained at 48 and 64 h postinfection (p.i.), respectively, to compare the ability of the viral antisera to detect virus-induced surface and intracellular antigens. Glutaraldehyde, a fixative known to retain membrane integrity, was used to fix the cells examined for surface reactions, and Formol buffered acetone, known to disrupt membranes, was used to expose intracellular antigens (3, 11) (Fig. 5 and 6). The foci of infected cells, both TN-368 and IPLB-SF-21, clearly stained much more intensly when acetone fixed than when glutaraldehyde fixed, as expected. The glutaraldehyde-fixed cells showed staining around the cell perimeters, characteristic of surface staining (3) with both anti-LOVAL and anti-BV sera. The surface staining was more intense with cells at the outer perimeters of the foci (which contained no polyhedra) than with the centrally located cells containing polyhedra. Many more of the acetone-fixed TN-368 cells in the prepolyhedra stage of infection (and earlier) stained with the anti-BV serum than the anti-LOVAL serum at comparable antiserum dilutions (Fig. 6).

DISCUSSION

Immunoblotting, first reported by Towbin et al. (17), is a derivative of the Southern blotting technique and has in common the tremendous power of identifying and locating specific antigenic determinants, or epitopes (when used in conjunction with monoclonal antibodies), as specific nucleic acid sequences are detected in Southern blots. When used in conjunction with conventional antisera, cross-reactions between viruses and viral phenotypes can be demonstrated and the protein components involved can be identified. Because immunoblotting is the composite product of other techniques and reagents, some of the problems inherent in the use of those techniques and reagents are necessarily reflected. For example, the LOVAL blots (Fig. 1 and 2) showed the problem of protein quantity for gel and blot analysis; loading enough virus to detect certain protein bands results in the merging and smearing of other bands, as well as differences in staining intensity, which may cause difficulty in interpretation. The problem of unequal protein component concentrations on the blot may be compounded (or reduced) by differences in the antigenicity of the components, both as immunogens and antigens. The

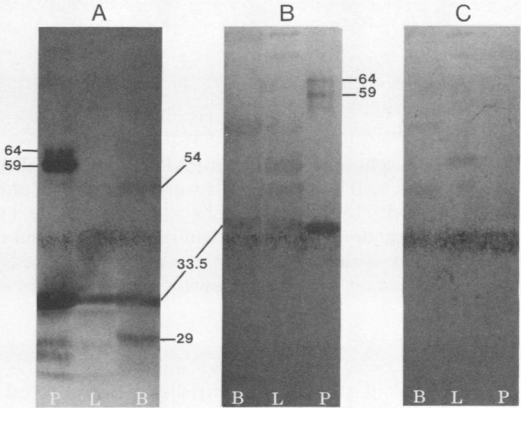

FIG. 4. Immunoblots of polyhedrin (P), LOVAL (L), and BV (B) from (A) 10% and (B and C) 12.5% SDS-polyacrylamide gels. Blot was treated with (A) conventional rabbit antiserum to polyhedrin, (B) monoclonal antibody to polyhedrin, or (C) normal rabbit serum.

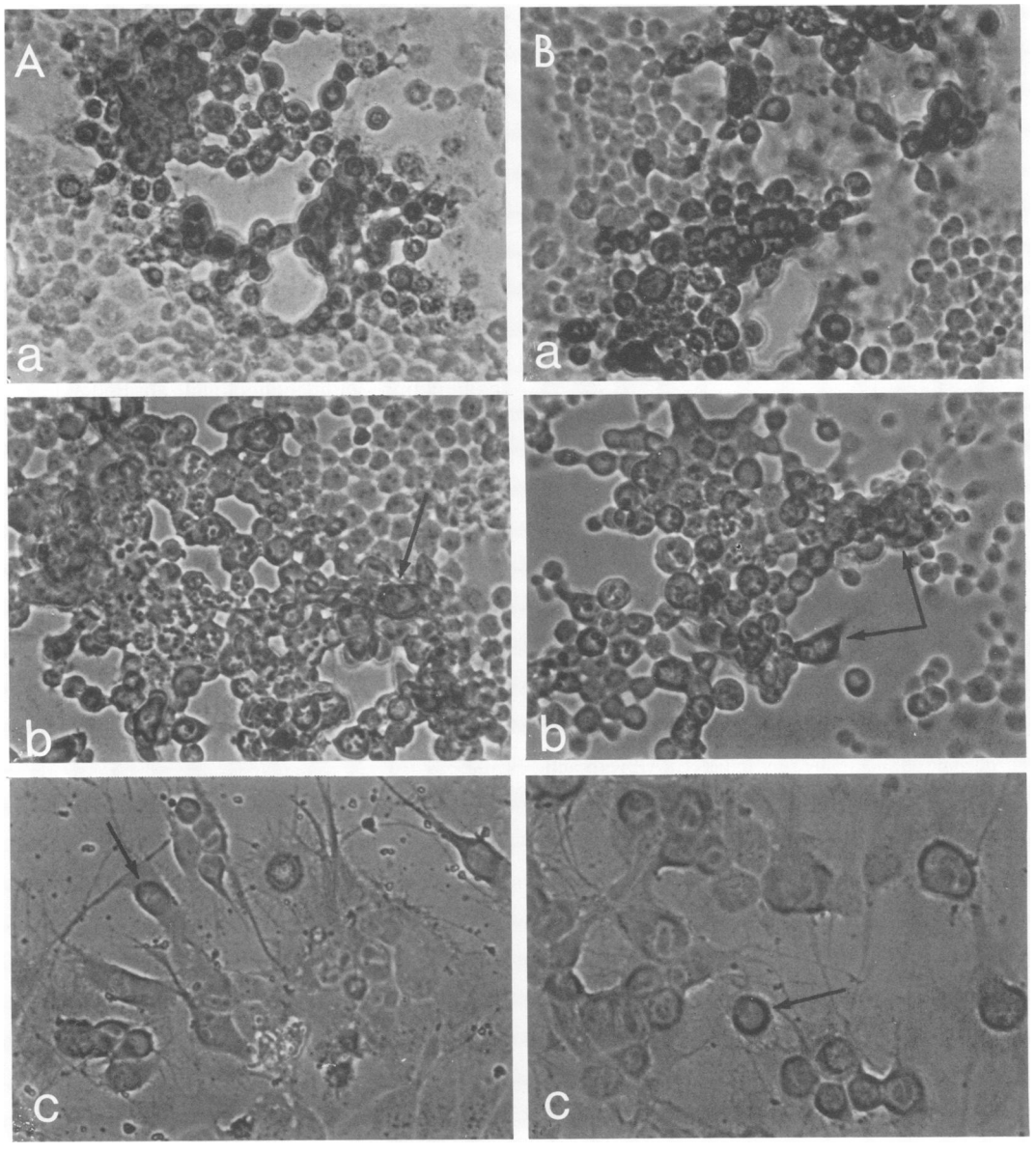

FIG. 5. Light micrographs of PAP-stained foci of AcNPV-infected IPLB-SF-21 cells ≤64 h p.i. (a and b) and TN-368 cells ≤40 h p.i. (c). Cells were stained with antiserum to BV (A) or to LOVAL (B) diluted at 1:200. Cells were fixed with buffered Formol acetone to expose intracellular antigens (a) or with 4% glutaraldehyde to allow antiserum reaction with surface antigens only (b and c). Surface staining was associated primarily with cells at the periphery of the focus (arrows) when cells at the center contained polyhedra (b). Cells in row c clearly show ring-type staining patterns (arrows) characteristic of surface staining.

antigenicity of the components can be affected further by the denaturing conditions of SDS-PAGE. With AcNPV, an additional problem is the complete and distinct separation of all the protein components so that minor proteins are not masked by intense reactions with major proteins.

In general, the reaction intensity of the blotted LOVAL proteins was similar with both anti-LOVAL and anti-BV sera (Fig. 3). The reaction intensity of the BV blots, however, was greater with anti-BV serum. These observations were in accordance with ELISA results, which indicated that the limits of sensitivity of the two antisera

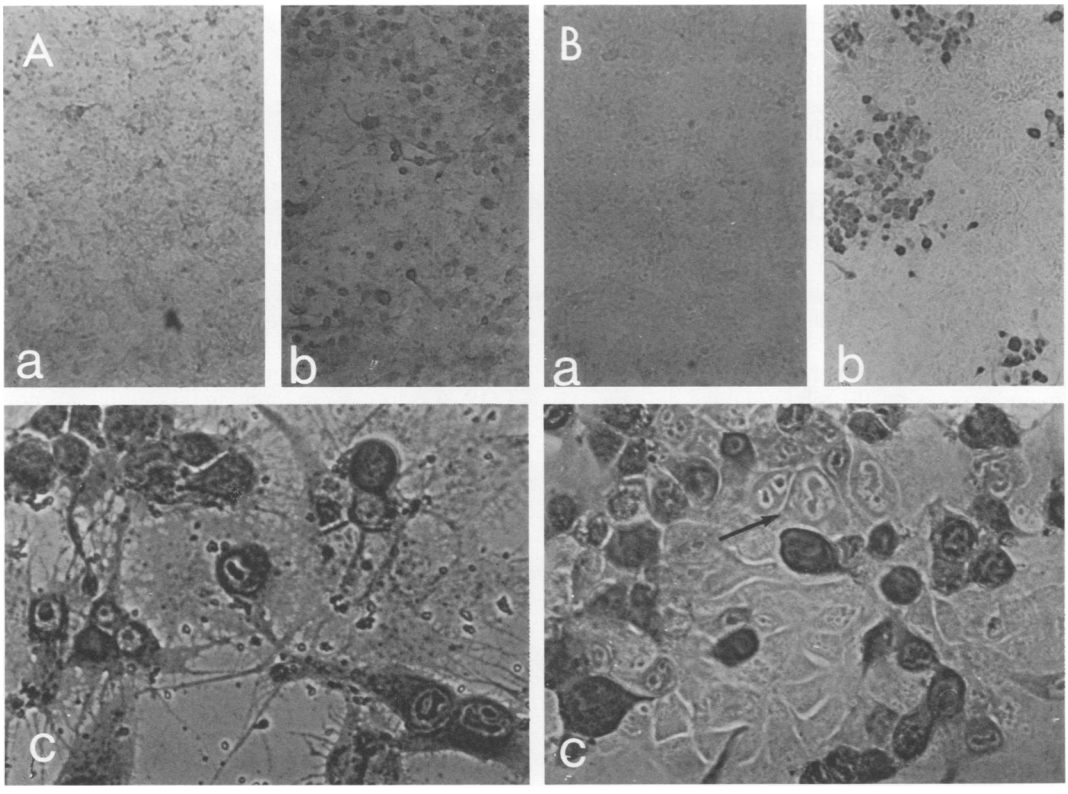

FIG. 6. Light micrographs of PAP-stained foci of AcNPV-infected (b and c) and uninfected (a) TN-368 cells fixed with buffered Formol acetone. (A and B) Duplicate slide cultures stained with antiserum to BV (Ab, Ac) or to LOVAL (Bb, Bc) diluted 1:200. Many more cells showed staining in Ab than in Bb above their respective backgrounds (Aa and Ba). Higher magnification of a region shown in Bb is seen in Bc, and some infected cells in the prepolyhedra stage of cytopathic effect were not stained (arrow). No such cells were evident among those stained with antiserum to BV (Ac).

were about the same for LOVAL, but for BV the anti-BV serum was about fivefold more sensitive (unpublished data).

The MW 92,500, 78,000, 62,500, 54,000 and 42,500 proteins of both LOVAL and BV reacted intensely with both homologous and heterologous antisera, although the reactions with the 62,500- and 42,500-MW proteins of BV were much more extensive with both antisera than the comparable LOVAL protein reactions, probably reflecting the relatively greater abundance of these proteins in BV (Fig. 1 and 2). The comparable MWs and the similar reciprocal reaction patterns suggests that these five proteins may be the same or very similar for both phenotypes. Further, since anti-LOVAL serum does not neutralize BV infectious activity in vitro as does anti-BV serum (24), it could be speculated that these proteins, one of which is a glycoprotein (5), are not involved in BV neutralization. This must be substantiated, however.

Two additional LOVAL proteins, with MWs of 46,000 and 36,000, gave intense reactions with anti-LOVAL serum but not with anti-BV serum. A very slight reaction occurred with a 46,000-MW BV protein with anti-LOVAL serum that appeared to be distinct from an overlapping reaction in that region, probably resulting from trailing of the neighboring copious and strongly reacting 42,500-MW protein.

Anti-LOVAL serum reacted in a similar way to another BV protein of MW 65,000 that was apparent as a distinct band under the trailing reaction of the major MW 62,500 protein (Fig. 3). The reaction with the anti-BV serum was not distinct and was contiguous with the 62,500-MW protein major reaction. Unlike the 46,000-MW protein, no LOVAL 65,000-MW counterpart could be detected. This phenomenon of an antiserum reacting with a protein of the heterologous phenotype that had no evident MW counterpart in the homologous phenotype occurred in at least two other instances: the anti-LOVAL serum reaction with the BV 102,000-MW pro-

tein, and the anti-BV serum reaction with the LOVAL 32,000-MW protein (Fig. 3). These observations indicated that these proteins are shared by both viral phenotypes but in different, modified versions, or that different genes code for separate but serologically related proteins for the two phenotypes.

Both BV and LOVAL had prominent 36,000- and 34,000-MW proteins that were recognized to a considerably greater extent by homologous than by heterologous antisera, indicating that these proteins of similar MW were distinct for each phenotype.

The LOVAL 45,000-MW protein, present as a major protein on the silver-stained gel (Fig. 1), did not stain on the blot with antiserum to LOVAL. Possibly this protein was either a poor immunogen or denatured beyond immunological recognition or both.

With some specific proteins, most clearly with the BV 48,000-MW protein, the PAP method of staining resulted in a light-on-dark banding pattern. The reason(s) for this is not understood, although in some cases it could be due to competition between immunoreactive and nonreactive proteins of the same MW. The nonreacting protein could be a different protein altogether, or it could be partially denatured. The nonreaction of the LOVAL 46,000-MW protein with anti-BV serum looked similar to the center milkiness of the light-on-dark banding. Another possibility is that with high concentrations of certain proteins, the colored diaminobenzidene tetrahydrochloride polymeric oxidation product precipitate did not adhere tightly due to chemical incompatibilities and was lost during the final rinses.

Smith and Summers (14) found that with a radioimmunoassay blotting technique, only 4 BV proteins reacted with anti-LOVAL serum, whereas I found that 18 reacted. Differences in reagents, techniques, and experimental conditions could account for this discrepancy. My results further conflicted with those of Smith and Summers in that they reported detecting no protein immunologically related to and comigrating with polyhedrin in their LOVAL blots. I detected such a reaction not only with LOVAL, but with BV as well. Normal serum did not react with polyhedrin or with the BV or LOVAL 33,500-MW comigrating proteins, but a monoclonal antibody to polyhedrin did. Clearly a polyhedrin-like protein was present in both the LOVAL and BV preparations. Although contamination of the LOVAL preparation with polyhedrin could not be ruled out (2), it was much more questionable in the case of BV. Also, the reaction of the 54,000- and 29,000-MW proteins with anti-polyhedrin serum in the BV blots, but not in the LOVAL blots, detracted from the simple contamination explanation for the BV results. Although polyhedrin is generally thought of as a late protein (4, 5, 25), it has been detected in AcNPV-infected *S. frugiperda* cells at 10 h p.i., just before the appearance of newly synthesized virus (4). Further, its precursor has been detected as early as 6 h p.i. (4), so that the association of polyhedrin with BV is not temporally inconsistent, just curious. Whether the BV 54,000- and 29,000-MW proteins are related to polyhedrin as precursor and cleavage product remains to be seen.

Considering the report that AcNPV LOVAL contains only three or four capsid proteins (15) and that 18 BV proteins that reacted with anti-LOVAL serum were found in this study, it is not surprising that anti-LOVAL serum recognized virus-induced cell surface antigens, which, presumably, were destined to become BV envelope components. The implications are that immunologically similar proteins are transported from the cytoplasm both to the nucleus, where they are incorporated into the newly synthesized LOVAL envelopes, and to the plasma membrane, where they are incorporated into the envelopes of BV.

It was observed that, in general, the most intense surface staining was associated with infected IPLB-SF-21 (Fig. 5) and TN-368 (not shown) cells at the periphery of the foci. This observation was expected, since in AcNPV-infected TN-368 cells budding has been found to be associated with the morphologically recognizable prepolyhedra stage of infection and begins at about 8 h p.i. (22, 24). The staining results also indicated that for both AcNPV-infected IPLB-SF-21 and TN-368 cells, viral antigen is not substituted into the plasma membrane after budding stops and polyhedra are formed.

The antiserum to BV not only reacted with close to 100% of the TN-368 cells in the morphologically recognizable prepolyhedra stage of infection, but also with cells apparently morphologically unaltered and therefore infected for 8 h or less (24). Anti-LOVAL serum, on the other hand, used at the same concentration did not detectably react with a considerable number of cells in the prepolyhedra stage of infection, much less with cells infected for less than 8 h. These observations, coupled with the ELISA results mentioned above, indicate that the antiserum to BV was reacting with early antigens that were relatively more abundant in, if not exclusive to, BV. The best candidate for the earliest antigen recognized, made before the onset of the prepolyhedra morphological changes or before 10 h p.i. (24), and represented abundantly in BV and less abundantly in LOVAL is the 48,000-MW protein. Three independent radioisotope labeling studies have detected the early production (before 8 h p.i.) of a

protein in this MW region (45,000, 46,000, and 48,000) (4, 5, 25). With the advent of the prepolyhedra morphological changes at 8 to 10 h p.i., antibodies to the proteins of MWs 62,500 and 42,500, which apparently are considerably more abundant in the BV than in the LOVAL antiserum, could also be responsible for the more extensive staining observed with the anti-BV serum at this time.

The immunoblot and PAP assay approach to comparing the relatedness of structural proteins of the two AcNPV phenotypes yielded some interesting information complementary to that derived from previous studies, which depended largely on MW estimates for structural component identification. In the absence of sequence data, antigenicity is an important identification character for proteins, especially when used in conjunction with MW. It is clear, however, that the complexity of the two phenotypes is such that the use of more specific serological probes, i.e., monospecific sera or monoclonal antibodies, in conjunction with this approach would yield a great deal more information on the identity and possible processing of structural components, as well as on their time of synthesis and their final position in the viral nucleocapsid or envelope.

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