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## High Resolution Two-Dimensional Gel Electrophoresis of Structural Proteins of Baculoviruses of *Autographa californica* and *Porthetria (Lymantria) dispar*

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Received July 14, 1982; accepted November 9, 1982

The structural polypeptides of baculoviruses of *Autographa californica* (AcMNPV) and *Porthetria dispar* (PdMNPV) were analyzed by two-dimensional (2-D) gel electrophoresis. Purified proteins were solubilized in urea-NP40 mix and separated by isoelectric focusing in the first dimension; electrophoresis in the presence of sodium dodecyl sulfate (SDS) separated proteins by molecular weight in the second dimension. Eighty-one acidic polypeptides ranging in molecular weight from 13,500 to 86,000 Da were resolved in AcMNPV enveloped virions. The predominant polypeptide had a molecular weight of 41,500 and was considered to be the major capsid protein. Nucleocapsids from AcMNPV were resolved into 64 polypeptides. At least 11 of the polypeptides, including most of the high molecular weight proteins, that were not resolved in nucleocapsids were considered to be envelope proteins. For PdMNPV enveloped virions, there were 95 acidic polypeptides ranging in molecular weight from 13,500 to 85,500. The predominant polypeptide had a molecular weight of 46,500 Da. Polyhedral proteins (polyhedrin) isolated from protease-inactivated polyhedra and separable into a single major polypeptide (approx. 31,000) on one-dimensional SDS-polyacrylamide gel electrophoresis were resolved into six polypeptides for both viruses. All six polyhedrin polypeptides had the same molecular weight, but their isoelectric points ranged from pH 5.3 to 5.9 for AcMNPV and from pH 5.7 to 6.2 for PdMNPV. These six polypeptides were also detected when protease-inactivated or noninactivated whole polyhedra were analyzed directly by 2-D electrophoresis. It is assumed that not all the observed baculovirus polypeptides were unique species. Some proteins, especially the polyhedrin polypeptides, appeared to be related and had altered mobilities as a consequence of post-translational modifications.

### INTRODUCTION

Baculoviruses are rod-shaped, double-stranded DNA viruses which have shown great potential as entomopathogenic bio-control agents. Baculovirus virions are either nonoccluded or occluded in a proteinaceous inclusion body. These viruses are classified into two major groups: nuclear polyhedrosis viruses (NPVs) and granulosis viruses. NPVs contain a number of enveloped nucleocapsids per inclusion body (polyhedron); some NPVs consist of either a single nucleocapsid (SNPV) or one to

several nucleocapsids (MNPV) per envelope. Granulosis viruses contain a single enveloped nucleocapsid per inclusion body (granule or capsule).

Several one-dimensional (1-D) electrophoretic methods, mainly on acrylamide gels in the presence of sodium dodecyl sulfate (SDS), have been used for analysis of NPV polyhedral protein (polyhedrin) and virion polypeptides (Young and Lovell, 1973; Padhi *et al.*, 1974; Kozlov *et al.*, 1975; McCarthy and Liu, 1976; Cibulsky *et al.*, 1977a, b; Eppstein and Thoma, 1977; Har-rap *et al.*, 1977; Summers and Smith, 1978; Maruniak *et al.*, 1979; Zethner *et al.*, 1979;

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Singh *et al.*, 1980; Maskos and Miltenburger, 1981). Generally, polyhedrin has been found to consist of a single major polypeptide of approximately 25,000–33,000 Da while virions contain 11–25 polypeptides ranging from 10,000 to 160,000 in molecular weight. Recently, several workers have utilized autoradiography to improve the resolution obtained with 1-D SDS-polyacrylamide gel electrophoresis (PAGE) and have detected 22–36 polypeptides in *Autographa californica* MNPV-infected cells (Carstens *et al.*, 1979; Dobos and Cochran, 1980; Wood, 1980; Maruniak and Summers, 1981) and virions isolated from the extracellular fluid of infected cells (Vlak, 1979). However, almost all of these cases involved major proteins; minor components, or even relatively abundant ones, may not be distinguished if their molecular weight is similar to that of one of the major components.

In the high resolution, two-dimensional (2-D) gel electrophoretic technique developed by O'Farrell (1975), proteins are separated by isoelectric focusing in the first dimension and according to molecular weight by SDS electrophoresis in the second dimension. The technique can be used for identification of genetically altered or modified polypeptides that are separable by minor charge differences, and is capable of resolving over 1,000 proteins. 2-D gel electrophoresis has been applied extensively for analysis of a number of proteins from complex biological systems including human tissues (Anderson and Anderson, 1979), bacteria (Phillips *et al.*, 1980), and animal viruses (O'Farrell and Goodman, 1976; Essani and Dales, 1979; Churchill and Radloff, 1981; Haarr and Marsden, 1981; Kilpatrick and Rouhandeh, 1981; Oie and Ichihashi, 1981; Siddell *et al.*, 1981). To the best of our knowledge there are no reports on use of this technique with baculoviruses.

This paper demonstrates the application of 2-D gel electrophoresis to proteins of virions, polyhedrins, and whole polyhedra from NPVs of *Autographa californica* (AcMNPV) and *Porthetria dispar* (PdMNPV).

## MATERIALS AND METHODS

### Chemicals

Ampholines were purchased from LKB Instruments, Inc., Rockville, Maryland. Urea, SDS,  $\beta$ -mercaptoethanol, acrylamide, bisacrylamide, ammonium persulfate, *N,N,N',N'*-tetramethylethylenediamine (TEMED), agarose, Coomassie blue, and bromophenol blue of electrophoresis purity grade were obtained from Bio-Rad Laboratories, Richmond, California. Nonidet-P40 (NP40) was from BDH Chemicals Ltd., Poole, England. Tris base, molecular weight marker proteins, glycerin, and Ficoll were purchased from Sigma Chemical Company, St. Louis, Missouri. Sepharose 2B was obtained from Pharmacia Fine Chemicals, Piscataway, New Jersey. All other chemicals were from Fisher Scientific Company, Atlanta, Georgia.

### Viruses

AcMNPV was isolated from infected *Trichoplusia ni* larvae kindly provided by T. R. Shieh, Sandoz, Inc. PdMNPV was purified from the commercial preparation "Gypchek" donated by F. B. Lewis, USDA Forest Service, Hamden, Connecticut.

Polyhedra were isolated according to procedures described earlier (Singh *et al.*, 1979). Gypchek was suspended in 0.01 M Tris, pH 7.8, and, as with AcMNPV larval homogenate, was subjected to several cycles of low speed centrifugation. The crude polyhedral suspension was purified on a 40–60% (w/w) sucrose gradient, treated with 4.0 M urea and 1% SDS, sonicated for 4 min, and repurified by zonal centrifugation in a Beckman Model L5-75 ultracentrifuge equipped with Ti-15 zonal rotor.

### Virion Purification

Virions were released from polyhedra using methods described by Summers and Smith (1978) with certain modifications. Lyophilized polyhedra were treated with 0.1 M  $\text{Na}_2\text{CO}_3$ , 0.01 M EDTA, 0.17 M NaCl, pH 10.9 (5 mg/ml), with gentle stirring for 60 min at 0–4°. The dissolution mixture was diluted twofold with distilled water,

and centrifuged at 4,000 *g* for 10 min at 4° to sediment undissolved polyhedra. Virions in the supernatant were pelleted by centrifugation at 55,000 *g* for 45 min at 4°. The pellet was suspended in 0.1 *M* Tris, 0.01 *M* EDTA, pH 7.8, and layered onto a 5–30% (w/w) Ficoll gradient prepared in the same buffer (Bell and Orlob, 1977). The gradient tubes were centrifuged at 25,000 rpm in an SW 27 rotor for 90 min at 4°. Virus bands were removed, diluted, and washed with distilled water by centrifugation at 4°. In some cases, the virions were further purified by Sepharose 2B column chromatography as described below. The final washed pellet (enveloped virions) was either resuspended in a small amount of distilled water (for electron microscopy) or treated with solubilization buffer for isoelectric focusing.

#### *Preparation of Nucleocapsids*

Nucleocapsids were produced from AcMNPV enveloped virions by a modification of the procedure described by Harap *et al.* (1977). In a preliminary test, the virion preparation was either sonicated for 5–10 min at 30–60-sec intervals (Singh *et al.*, 1979) or adjusted to a final concentration of 500  $\mu\text{g}$  protein/ml with 0.5, 1, or 2% NP40 in 0.05 *M* Tris, 0.03 *M* NaCl, pH 7.6, and incubated for 30 min at 30°. Attempts were made to centrifuge the sonicate and virus-NP40 suspensions on 2.5–40% (w/w) Ficoll and 5–50% (w/w) sucrose gradients at 25,000 rpm in an SW 27 rotor for 30, 60, or 90 min at 4 or 20°. With all of these methods, nucleocapsids did not separate into a discrete band and tended to migrate to the bottom of the gradient tube, therefore, the procedure was modified as follows: Virions were incubated at a final concentration of 500  $\mu\text{g}$  protein/ml in 1% NP40 for 30 min at 30°. The suspension was diluted fivefold with distilled water and centrifuged at 55,000 *g* for 45 min at 4°. The pellet was suspended in 0.01 *M* borate buffer, pH 9.0, and passed through a 1.6  $\times$  40-cm column packed to a height of 25 cm with Sepharose 2B (Bell and Orlob, 1977). Samples were applied at

the top of the column previously equilibrated in borate buffer. Two-milliliter fractions, monitored at 254 nm, were collected at a flow rate of 20 ml/hr, and concentrated by centrifugation at 100,000 *g* for 30 min at 4°. The final washed pellet (nucleocapsids) was either suspended in water (for electron microscopy) or solubilized in urea-NP40 mix (see below) for isoelectric focusing. Nucleocapsids were not prepared from PdMNPV for lack of sufficient quantity of virions.

#### *Preparation of Polyhedrin*

Protease inactivation of polyhedra and purification of polyhedrin were basically as described by Summers and Smith (1978). Dissolution mixtures were diluted twofold with distilled water and centrifuged at low (5,000 *g*, 10 min) and high (55,000 *g*, 45 min) speeds at 4°. Polyhedrin in the supernatant was collected by precipitation at pH 5.5 (Longworth *et al.*, 1972) and subsequent centrifugation at 5,000 *g* for 30 min at 4°. The precipitate was resuspended in 0.01 *M* Na<sub>2</sub>CO<sub>3</sub>, 0.05 *M* NaCl, pH 9.4 (Scott *et al.*, 1971), dialyzed against the same buffer (pH 9.0) overnight, and, if necessary, subsequently concentrated by ultrafiltration (Amicon, Diaflow membrane, PM 10). The final preparation was solubilized either in SDS mix for 1-D SDS-PAGE or urea-NP40 mix (see below) for isoelectric focusing.

#### *Protein Determinations*

Protein determinations were made by the method of Bradford (1976) using the dye reagent and gamma globulin standard supplied by Bio-Rad Laboratories.

#### *Electron Microscopy*

Virion and nucleocapsid preparations were stained with 1% phosphotungstic acid, pH 3.0, and examined with a Philips 300 electron microscope (Singh *et al.*, 1979).

#### *Solubilization of Samples*

In a preliminary test, AcMNPV virions were treated with four solubilization buffers to determine the most suitable system:

- (1) Virions were suspended in a 0.0625 M Tris solution, pH 6.8, containing 2% SDS, 5% mercaptoethanol, and 10% glycerol ("SDS mix"), heated at 100° for 5 min, and cooled on ice.
- (2) Virions were suspended in SDS mix containing 1% NP40 ("SDS-NP40 mix") as described by Kilpatrick and Rouhandeh (1981) and boiled and cooled as above.
- (3) Virions were suspended in a lysis buffer (O'Farrell, 1975) modified to contain 4% NP40 ("urea-NP40 mix"; Anderson *et al.*, 1979), and subjected to five cycles of freezing and thawing.
- (4) Virions were suspended (at three times the concentration used in the other three mixes) in SDS mix, boiled and cooled, and diluted with 2 vol of a modified urea mix containing 8% NP40 ("SDS-urea-NP40 mix") as described by Ames and Nikaido (1976).

Routinely, samples were solubilized at a concentration of 1–2.5  $\mu\text{g}$  (polyhedrin) or 3–5  $\mu\text{g}$  (enveloped virions, nucleocapsids, or polyhedra) protein/ $\mu\text{l}$  of solubilization buffer. Solubilized samples were electrophoresed the same day or within 72 hr of storage at  $-80^\circ$ . Molecular weight marker proteins were solubilized in SDS mix, stored in small aliquots at  $-80^\circ$ , and used over a period of 3–4 months.

#### Isoelectric Focusing

The first dimension isoelectric focusing was carried out as described by O'Farrell (1975) with some modifications. Gels (105 mm in length) were prepared in 125  $\times$  2-mm (internal diameter) glass tubes. The focusing mixture consisted of 9.5 M urea, 4% acrylamide, 2% NP40, and 2% ampholines comprised of pH 4–6, 6–8, and 3.5–10 in the ratio of 2:2:1 (Ames and Nikaido, 1976). Ammonium persulfate and TEMED were used to initiate polymerization. Virus samples (25–150  $\mu\text{g}$  protein) were applied at the top of the gel and subjected to isoelectric focusing at 300 V for 18 hr followed by 400 V for 1.5 hr (Jesse Edwards, personal communication). Focused gels were gently shaken in equilibration buffer

(O'Farrell, 1975) for 10–30 min, and stored at  $-80^\circ$  (maximum 1 month) until used in second dimension electrophoresis. The pH gradient of focused gels was monitored by cutting control gels into 5- to 10-mm pieces. Each piece was placed in a test tube containing 1.5 ml degassed distilled water and allowed to equilibrate overnight at room temperature; pH was measured with a pH meter. The gradients typically were between pH 3.8 and 6.8 (Fig. 1).

#### Second Dimension SDS-PAGE

The methods for SDS-PAGE were patterned after those of O'Farrell (1975). A Bio-Rad Model 220 slab electrophoresis cell was used throughout. All separations were performed on 8–20% acrylamide gradient gels measuring 140  $\times$  110  $\times$  1.5 mm. The stacking gel was approximately 10 mm in height. The cathode and anode electrode buffers were 0.04 M boric acid, 0.08 M Tris, 0.1% SDS, pH 8.5, and 0.375 M Tris-HCl, pH 8.8, respectively (Neville, 1971). A focused gel was quickly thawed, loaded onto the slab gel as described by Anderson *et al.* (1979), and immediately layered with

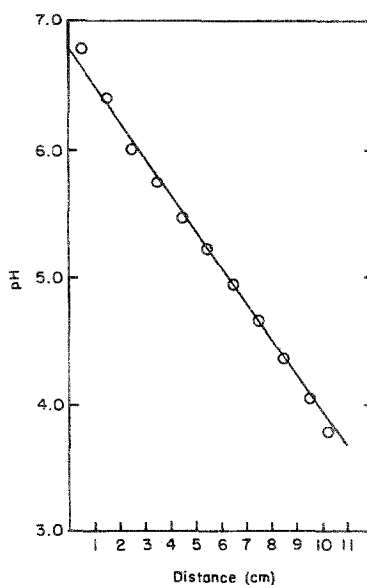


FIG. 1. pH gradient for the isoelectric focusing gel. The zero point on the abscissa represents the basic (top) end of the gel.

warm 1% agarose dissolved in equilibration buffer. Molecular weight standards were applied to a well made in agarose on the left side of the slab (Ames and Nikaïdo, 1976) and the gels were electrophoresed at 20 mA for 60 min followed by 40 mA until the dye front reached the bottom of the slab. The total running time was approximately 4 hr 45 min. At the end of a run, the separating gels were stained with 0.1% Coomassie blue and destained with several changes of methanol-acetic acid-water solutions (Anderson *et al.*, 1979). Destained gels were either dried with a Bio-Rad Model 224 gel slab dryer or photographed with a Polaroid MP<sub>4</sub> Land camera using Kodak contrast process pan film (Anderson *et al.*, 1979).

## RESULTS

### *Solubilization of Virus Samples*

Effectiveness of the four solubilization buffers was judged by the number and overall resolution of individual polypeptides on the second dimension gel. The 2% SDS with or without NP40 and urea did not completely solubilize AcMNPV virions, resulting in fewer and poorly resolved polypeptides and excessive streaking over the right-hand portion of the gel (data not shown). The urea-NP40 mix consistently gave better overall separation and was therefore adopted for use with all virus components. Although several proteins did not enter the focusing gel, no attempt was made to centrifuge the solubilized sample.

### *Virion Polypeptides*

Following density gradient centrifugation, virions separated into six peaks typical of MNPV (Singh *et al.*, 1979). Peaks 1-6 were routinely collected, and electron microscopic examination showed predominantly intact, undegraded, enveloped virions (>95%). The virion yield from AcMNPV polyhedra was approximately five times higher than that from PdmNPV polyhedra.

A total of 81 polypeptides ranging in

molecular weight from 13,500 to 86,000 was separated from AcMNPV enveloped virions by 2-D electrophoresis (Figs. 2, 3). The most abundant polypeptide had a molecular weight of 41,500. A major protein with a molecular weight of 40,000-42,000 in AcMNPV virions has been reported by several investigators (Vlak, 1979; Dobos and Cochran, 1980) and was identified by Smith and Summers (1981) as a capsid protein. Other major proteins had molecular weights of 15,000, 16,700 (two separate polypeptides), 20,000, and 49,500. None of the virion polypeptides appeared to be polyhedrin. An intensely staining poly-

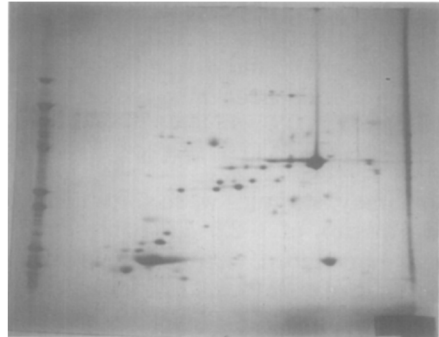


FIG. 2. Two-dimensional gel electrophoresis of AcMNPV enveloped virions. Virions, released by alkaline dissolution of polyhedra for 60 min at 0-4°, were purified on 5-30% (w/w) Ficoll gradient. Virus samples were solubilized in urea-NP40 mix by repeated freezing and thawing. A 150- $\mu$ g protein sample was applied at the top of the cylindrical gel and focused for a total of 6,000 voltage hr at room temperature. Focused gels were briefly equilibrated and then exposed to separation in the second dimension on 8-20% SDS-acrylamide gradient gel at 20 mA for 1 hr followed by 40 mA until the tracking dye reached the bottom of the gel. Molecular weight marker proteins (phosphorylase 93,000, bovine serum albumin 66,000, egg albumin 45,000, carbonic anhydrase 30,000, trypsinogen 24,000, lactoglobulin 18,400, and lysozyme 14,300) were placed in a well in the agarose overlay and electrophoresed along with the first dimension gel. At the end of run, separating gels were stained with 0.1% Coomassie blue, destained in methanol-acetic acid-water, and photographed with Polaroid MP<sub>4</sub> Land camera using Kodak contrast process pan film. The molecular weights of virus polypeptides were estimated by a comparison of their relative mobilities with those of known marker proteins.

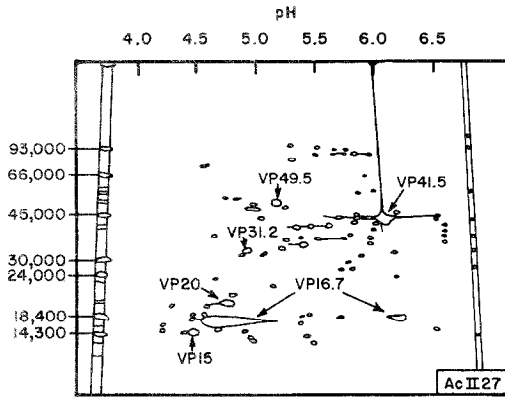


FIG. 3. Diagram of the gel in FIG. 2. Molecular weights of major virus polypeptides (VP) are indicated as calculated molecular weight ( $\times 10^3$ ).

peptide had the same molecular weight as AcMNPV polyhedrin (31,200) but a different isoelectric point (see next section). An AcMNPV virion polypeptide with molecular weight similar to polyhedrin has also been reported by Smith and Summers (1981) who have shown it to be immunologically unrelated to polyhedrin. In general, our estimates of the molecular weight of the major polypeptides of AcMNPV virions were in agreement with those reported in the literature. None of the polypeptides resolved by 2-D electrophoresis had molecular weights above 86,000. Possibly, some of the higher molecular weight ( $>86,000$ ) proteins reported by other investigators for AcMNPV (Summers and Smith, 1978; Vlcek, 1979; Dobos and Cochran, 1980; Maruniak and Summers, 1981) were aggregates which were dissociated under the solubilization conditions used in this investigation.

There were 64 polypeptides in AcMNPV nucleocapsids (Figs. 4 and 5). The major proteins had molecular weights of 41,500, 19,800, and 16,500 (two polypeptides), which agreed generally with the molecular weights of corresponding virion proteins. It is unlikely that all of the 17 or so polypeptides that were present in virions but "missing" from nucleocapsids were envelope proteins. Lack of sufficient amounts of nucleocapsid samples prevented us from heavy-loading of focusing gels as was done

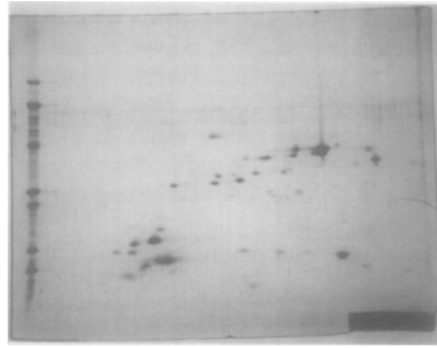


FIG. 4. Two-dimensional gel electrophoresis of AcMNPV nucleocapsids. Virions were released from polyhedra and purified by density gradient centrifugation as described in FIG. 2. Nucleocapsids (125  $\mu$ g protein), prepared by NP40 treatment of virions (500  $\mu$ g protein/ml of 1% NP40 in 0.05 M Tris, 0.03 M NaCl, pH 7.6, for 30 min at 30 $^\circ$ ) and purified by Sepharose 2B column chromatography, were solubilized and separated by 2-D electrophoresis as described in FIG. 2.

with virions. When nucleocapsid gels were compared with virion gels containing the same amounts of protein, the two were found to be quite comparable. This was expected since our nucleocapsid preparation contained small amounts of envelope material. Nevertheless, some polypeptides (e.g., one 23,000, one 38,500, one 49,500, and eight  $>50,000$ ) that were either present at

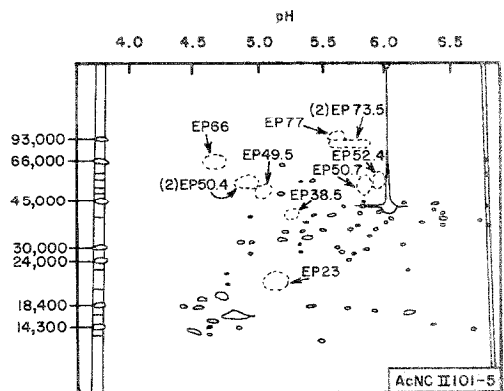


FIG. 5. Diagram of the gel in FIG. 4. Dotted circles represent approximate regions where a comparable virion gel contained 1 23,000, 1 38,500, 1 49,500, and 8  $>50,000$ -Da envelope polypeptides (EP).

a reduced intensity or were absent from nucleocapsid gels (Fig. 5) were considered to be envelope proteins. Harrap *et al.* (1977), using 1-D SDS-PAGE, showed that six polypeptides, including most of the large molecular weight proteins, were envelope proteins of three baculoviruses from the *Spodoptera* spp.

The 2-D electrophoresis profiles of PdMNPV virions showed a total of 95 polypeptides (Figs. 6, 7); molecular weight estimates of the major polypeptides were 46,500, 22,000, 16,100, 15,700, and 15,200. The predominant polypeptide (46,500) is probably the same as the 45,000-Da polypeptide reported by Maskos and Miltenburger (1981) and is likely a major capsid protein. There were at least nine polypeptides with a molecular weight of 47,500 Da, some of which were in a necklace-like connection. The differences observed in the 2-D profiles of AcMNPV and PdMNPV virions were not surprising since the two viruses were shown to be unrelated by genome analysis using restriction endonucleases (Smith and Summers, 1978), and by radioimmunoassay (Smith and Summers, 1981). DNA sequence homology studies have shown only 0.5–1% homologous regions between the genomes of these viruses (Jewell and Miller, 1980).

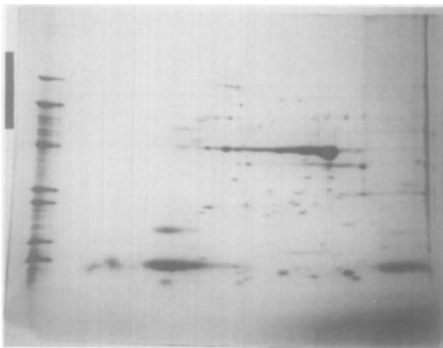


FIG. 6. Two-dimensional gel electrophoresis of PdMNPV enveloped virions. Virions were released from polyhedra and purified by density gradient centrifugation as described in FIG. 2. Virion samples (150  $\mu$ g protein) were solubilized in urea-NP40 mix and separated by 2-D electrophoresis as described in FIG. 2.

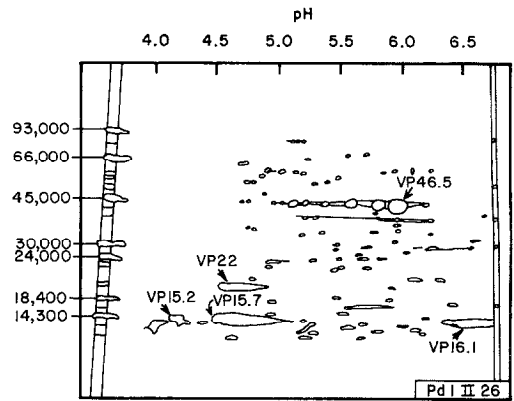


FIG. 7. Diagram of the gel in FIG. 6. Molecular weights of major virus polypeptides (VP) are indicated as calculated molecular weight ( $\times 10^3$ ).

### Polyhedrin Polypeptides

Polyhedrins purified from protease-inactivated polyhedra, solubilized in SDS mix by boiling, and subsequently separated by 1-D SDS-PAGE, consisted of a single major and several minor polypeptides (Figs. 8 and 9). The molecular weights of 31,200 and 30,600 for the major polypeptide of AcMNPV and PdMNPV, respectively, are consistent with the 28,000–33,000 molecular weight reported for the polyhedrins of these viruses (McCarthy and Liu, 1976; Summers and Smith, 1978; Zethner *et al.*, 1979; Dobos and Cochran, 1980; Maruniak and Summers, 1981; Maskos and Miltenburger, 1981; Smith and Summers, 1981). The presence of minor polypeptides of lower molecular weight as a result of polypeptide degradation (Summers and Smith, 1978) has been questioned since they were detected after protease-inactivation (Maskos and Miltenburger, 1981). We did not analyze SDS-solubilized polyhedrins by 2-D electrophoresis. However, when samples were solubilized in urea-NP40 mix, the lower molecular weight polypeptides did not appear on the second dimension gel nor were they trapped on top of the focusing gel. Recently, Siddell *et al.* (1981) reported that mobility of certain coronavirus proteins was altered when purified virus preparations were boiled in electrophoresis buffer. Also, heating of samples



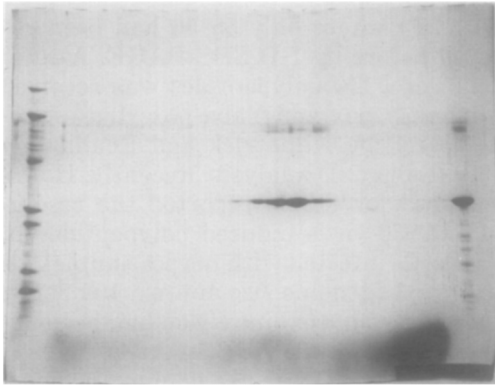


FIG. 8. One- and two-dimensional electrophoresis of AcMNPV polyhedral protein (polyhedrin). Polyhedra were heated at 70–72° for 2 hr and then equilibrated with 0.01 M HgCl<sub>2</sub> overnight to inactivate the protease. Polyhedrins were isolated by alkaline dissolution of polyhedra (0.1 M Na<sub>2</sub>CO<sub>3</sub>, 0.17 M NaCl, pH 10.8) for 10 min at 4° and subsequent high-speed centrifugation, isoelectric precipitation, and dialysis as described in the Materials and Methods section. Polyhedrin samples (25 μg protein) were solubilized in urea-NP40 mix and separated by 2-D electrophoresis as described in FIG. 2. Polyhedrin samples prepared in SDS mix (10 μg protein) were placed in a well at the opposite end of the molecular weight marker well on the SDS-acrylamide slab and electrophoresed along with the first dimension gel.

in the presence of SDS and subsequent addition of urea reportedly lead to artifactual spots on the second dimension gel (Esani and Dales, 1979).

The higher molecular weight (approximately 60,000) polypeptides are probably aggregates of the major polypeptide monomer since they have been shown to dissociate into polyhedrin monomer following additional reduction with mercaptoethanol (Summers and Smith, 1978) and their amino acid composition is similar to the monomer (Eppstein and Thoma, 1977). Several high molecular weight polypeptides were either mostly insoluble in urea-NP40 mix or their isoelectric points were below the pH range of the focusing gel as evidenced by their tendency to migrate to the bottom of the gel and the appearance of smear/streaking in the corresponding region on the second dimension gel (O'Farrell, 1975; O'Farrell and Goodman, 1976). Appearance as a smear may also reflect

charge heterogeneity or aggregation which restricts movement in the gel matrix (Hewick *et al.*, 1977).

Polyhedrin samples were solubilized in urea-NP40 mix for 2-D electrophoresis. Following isoelectric focusing, the cylindrical gels were either equilibrated and run on an SDS-slab or washed with trichloroacetic acid, stained, destained, and the distance migrated by each polypeptide was measured. This distance was used to estimate the approximate isoelectric point (pI) of polyhedrin polypeptides using the pH gradient curve (Fig. 1).

The major polyhedrin polypeptide from both baculoviruses consisted of six polypeptides all of which had the same molecular weight, but pI values ranged from pH 5.3 to 5.9 and 5.7 to 6.2 for AcMNPV and PdMNPV polyhedrins, respectively. There were approximately three major polypeptides in PdMNPV polyhedrin with pI values ranging from pH 5.9 to 6.2. The predominant polypeptide of AcMNPV polyhedrin had a pI of approximately 5.7, which is in close agreement with the reported value of 5.8 for *T. ni* SNPV (Eppstein and Thoma, 1977) and *Pieris brassicae* granulosis virus (Longworth *et al.*, 1972). The polyhedrins of AcMNPV and PdMNPV, although approximately the same size, have been shown to share only partial serological identity in immunodiffusion reactions (McCarthy and Lambiase, 1979).

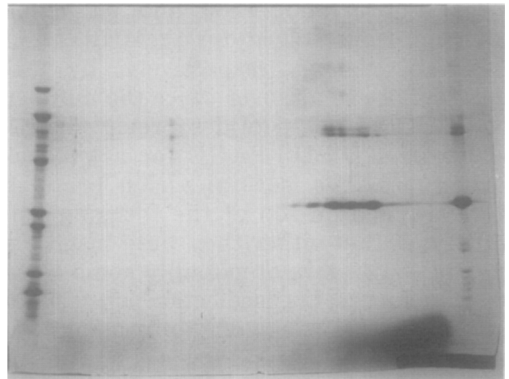


FIG. 9. One- and two-dimensional gel electrophoresis of PdMNPV polyhedrin. All experimental conditions were similar to those given in FIG. 8.

### *Polypeptides of Whole Polyhedra*

Whole polyhedra with and without protease inactivation were directly solubilized in urea-NP40 mix and analyzed by 2-D electrophoresis. The predominant proteins in both AcMNPV and PdMNPV were the six polypeptide units with molecular weights and pI values similar to those of purified polyhedrins.

Some of the virion proteins were also resolved; in general, more polypeptides were resolved in protease-noninactivated polyhedra than in protease-inactivated polyhedra (data not shown). We were able to resolve up to 30 virion polypeptides in AcMNPV polyhedra but only 5 in PdMNPV polyhedra.

### DISCUSSION

Some investigators have used high voltage electrophoresis (Cibulsky *et al.*, 1977a; Maruniak *et al.*, 1979) and 2-D electrophoresis consisting of a first dimension with agarose gel electrophoresis and a second dimension with SDS-PAGE or immunoelectrophoresis (Yamamoto and Tanada, 1979) for analysis of baculovirus proteins. O'Farrell's (1975) system, which uses two different, very high resolution separations, deals almost exclusively with individual gene products, simplifying genetic analysis of variants, and gives a total resolution of better than 0.1 charge unit and 1,000 Da for an average 50,000-Da protein (Anderson and Anderson, 1977).

The approximately 80 and 95 acidic polypeptides resolved for AcMNPV and PdMNPV virions, respectively, by 2-D electrophoresis are over twice the number of structural polypeptides previously resolved by 1-D SDS-PAGE for any baculovirus. Further, several virion proteins were trapped on top of the focusing gel, indicating that either they were insoluble in urea-NP40 mix or possibly some were basic in nature; i.e., isoelectric points were outside the pH range of the focusing gel.

Use of 2-D electrophoresis has also increased the resolution of structural polypeptides of several animal viruses. Essani and Dales (1979) separated 111 polypeptides in vaccinia virus (genome size 120-

130  $\times 10^6$ ) where only 55-56 had been resolved before by 1-D SDS-PAGE. A total of 115 and 140 polypeptides was resolved in monkey pox and Yaba pox viruses, respectively, by Kilpatrick and Rouhandeh (1981) using 2-D analysis. Recently, Haarr and Marsden (1981) reported the resolution of 230 virus-induced polypeptides in BHK cells infected with herpes simplex virus type 1 (genome size approx.  $100 \times 10^6$ ) where only approximately 50 had been detected before by 1-D SDS-PAGE.

Baculovirus DNAs are double-stranded, circular, supercoiled molecules with molecular weights of approximately 60-100  $\times 10^6$  Da (Burgess, 1977). Assuming an average gene size to be  $0.8 \times 10^6$  (Jewell and Miller, 1980), a baculovirus genome might consist of 75-100 genes. If all the >80 polypeptides resolved by 2-D electrophoresis were independently coded polypeptides, the DNA required to code for this number of virion polypeptides would exceed 75% of the genome. It is unlikely, however, that all observed polypeptides represent unique species. Some appeared to be related, but with altered mobilities perhaps due to post-translational modifications. Such post-translational modifications reportedly occur in AcMNPV (Carstens *et al.*, 1979) which contains 10-14 phosphoproteins, including the polyhedrin, and approximately six glycoproteins (Dobos and Cochran, 1980; Maruniak and Summers, 1981).

Phosphorylation, which adds one or more negative charges to the protein depending on the amino acid residue that is phosphorylated, is a likely explanation for the resolution of the AcMNPV and PdMNPV major polyhedrin polypeptide into multiple components. A polypeptide defined as a single phosphoprotein by the standard SDS-PAGE method is likely to be resolved into a cluster of spots in a necklace-like connection of slightly different pI values upon 2-D electrophoresis. For example, the major capsid protein of simian virus 40, called VP1 (molecular weight 47,000), consists of one major and five minor components (O'Farrell and Goodman, 1976). The minor components differ from the major form in molecular weight (by approx. 500) and pI (approx. range of pH 6.7-6.9) and

are the results of modification of the primary product of translation. Similar examples of a major phospho- or glycopolypeptide separating into multiple components are found in mouse c-type endogenous viruses (Chuat *et al.*, 1977), two plaque morphology variants of polyoma virus (Hewick *et al.*, 1977), herpes simplex virus type 1 (Haarr and Marsden, 1981), vaccinia virus (Oie and Ichihashi, 1981), and vesicular stomatitis virus (Hsu and Kingsbury, 1982). It is also likely that a small number of large molecular weight polypeptides may be the precursors (Oie and Ichihashi, 1981) of such a large number of baculovirus polypeptides. Some smaller polypeptides may thus be generated by proteolysis of these high molecular weight proteins (Vlak, 1979).

In conclusion, we have demonstrated the application of 2-D gel electrophoresis for baculoviruses and have resolved >80 proteins in AcMNPV and PdMNPV virions and six protein subunits in the major polyhedrin polypeptide. These numbers may be underestimates and one can anticipate that with further manipulation of the technique, e.g., use of nonequilibrium pH gradient electrophoresis (O'Farrell *et al.*, 1977), additional virion polypeptides will be demonstrated. The technique shows great potential for studies on baculoviruses such as determination of structural polypeptides *in vivo* and *in vitro*, differentiation of closely related strains, analysis of genetic mutants, and identification and classification.

#### ACKNOWLEDGMENTS

We thank Jesse Edwards of the Argonne National Laboratory for advice and criticism throughout this investigation. The schematic representations of the photographs were drawn by Mrs. Patricia A. Singh.

#### REFERENCES

- AMES, G. F. -L., and NIKAIIDO, K. (1976). Two-dimensional gel electrophoresis of membrane proteins. *J. Biochem.* **15**, 616-623.
- ANDERSON, L., and ANDERSON, N. G. (1977). High resolution two-dimensional electrophoresis of human plasma proteins. *Proc. Nat. Acad. Sci.* **74**, 5421-5425.
- ANDERSON, N. G., and ANDERSON, N. L. (1979). Molecular anatomy. *Behring Institute Symposium. Behring Inst. Mitt.* **63**, 169-210.
- ANDERSON, N. G., ANDERSON, N. L., and TOLLAKSEN, S. L. (1979). Operation of the iso-dalt system. *Argonne Nat. Lab. News Bull. ANL-BIM* **79-2**, 27.
- BELL, C. D., and ORLOB, G. B. (1977). Serological studies on virions and polyhedron protein of a nuclear polyhedrosis virus of the cabbage looper, *Trichoplusia ni*. *Virology* **78**, 162-172.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
- BURGESS, S. (1977). Molecular weights of lepidopteran baculovirus DNAs: Derivation by electron microscopy. *J. Gen. Virol.* **37**, 501-510.
- CARSTENS, E. B., TJIA, S. T., and DOERFLER, W. (1979). Infection of *Spodoptera frugiperda* cells with *Autographa californica* nuclear polyhedrosis virus. I. Synthesis of intracellular proteins after virus infection. *Virology* **99**, 386-398.
- CHUAT, J. C., LAPREVOTTE, L., BERNARD, C., and PILON, C. (1977). The isoelectric point of the p 30 polypeptide as a marker of mouse endogenous viruses. *J. Gen. Virol.* **38**, 169-173.
- CHURCHILL, M. A., and RADLOFF, R. J. (1981). Two-dimensional electrophoretic analysis of encephalomyocarditis viral proteins. *J. Virol.* **37**, 1103-1106.
- CIBULSKY, R. J., HARPER, J. D., and GUDAUSKAS, R. T. (1977a). Biochemical comparison of polyhedral protein from five nuclear polyhedrosis viruses infecting plusiine larvae (Lepidoptera: Noctuidae). *J. Invert. Pathol.* **29**, 182-191.
- CIBULSKY, R. J., HARPER, J. D., and GUDAUSKAS, R. T. (1977b). Biochemical comparison of virion proteins from five nuclear polyhedrosis viruses infecting plusiine larvae (Lepidoptera: Noctuidae). *J. Invert. Pathol.* **30**, 303-313.
- DOBOS, P., and COCHRAN, M. A. (1980). Protein synthesis in cells infected by *Autographa californica* nuclear polyhedrosis virus (Ac-NPV): The effect of cytosine arabinoside. *Virology* **103**, 446-464.
- EPPSTEIN, D. A., and THOMA, J. A. (1977). Characterization and serology of the matrix protein from a nuclear polyhedrosis virus of *Trichoplusia ni* before and after degradation by an endogenous proteinase. *Biochem. J.* **167**, 321-332.
- ESSANI, K., and DALES, S. (1979). Biogenesis of vaccinia: Evidence for more than 100 polypeptides in the virion. *Virology* **95**, 385-394.
- HAARR, L., and MARSDEN, H. S. (1981). Two-dimensional gel analysis of HSV type 1-induced polypeptides and glycoprotein processing. *J. Gen. Virol.* **52**, 77-92.
- HARRAP, K. A., PAYNE, C. C., and ROBERTSON, J. S. (1977). The properties of three baculoviruses from closely related hosts. *Virology* **79**, 14-31.
- HEWICK, R. M., WATERFIELD, M. D., MILLER, L. K., and FRIED, M. (1977). Correlation between genetic loci and structural differences in the capsid pro-

- teins of polyoma virus plaque morphology mutants. *Cell* 11, 331-338.
- HSU, C. -H., and KINGSBURY, D. W. (1982). NS phosphoprotein of vesicular stomatitis virus: Subspecies separated by electrophoresis and isoelectric focusing. *J. Virol.* 42, 342-345.
- JEWELL, J. E., and MILLER, L. K. (1980). DNA sequence homology relationships among six lepidopteran nuclear polyhedrosis viruses. *J. Gen. Virol.* 48, 161-175.
- KILPATRICK, D., and ROUHANDEH, H. (1981). The polypeptides of monkeypox virus. I. Analysis of the polypeptide synthesis of MPV by SDS-PAGE and by two-dimensional electrophoresis. *Virology* 110, 455-465.
- KOZLOV, E. A., SIDOROVA, N. M., and SEREBRYANI, S. B. (1975). Proteolytic cleavage of polyhedral protein during dissolution of inclusion bodies of the nuclear polyhedrosis viruses of *Bombyx mori* and *Galleria mellonella* under alkaline conditions. *J. Invert. Pathol.* 25, 97-101.
- LONGWORTH, J. F., ROBERTSON, J. S., and PAYNE, C. C. (1972). The purification and properties of inclusion body protein of the granulosis virus of *Pieris brassicae*. *J. Invert. Pathol.* 19, 42-50.
- MARUNIAK, J. E., and SUMMERS, M. D. (1981). *Autographa californica* nuclear polyhedrosis virus phosphoproteins and synthesis of intracellular proteins after virus infection. *Virology* 109, 25-34.
- MARUNIAK, J. E., SUMMERS, M. D., FALCON, L. A., and SMITH, G. E. (1979). *Autographa californica* nuclear polyhedrosis virus structural proteins compared from *in vivo* and *in vitro* sources. *Intervirology* 11, 82-88.
- MASKOS, C. B., and MILTENBURGER, H. G. (1981). SDS-PAGE comparative studies on the polyhedral and viral polypeptides of the nuclear polyhedrosis viruses of *Mamestra brassicae*, *Autographa californica*, and *Lymantria dispar*. *J. Invert. Pathol.* 37, 174-180.
- MCCARTHY, W. J., and LAMBLASE, J. T. (1979). Serological relationships among plusiinae baculoviruses. *J. Invert. Pathol.* 34, 170-177.
- MCCARTHY, W. J., and LIU, S. -Y. (1976). Electrophoretic and serological characterization of *Porthetria dispar* polyhedron protein. *J. Invert. Pathol.* 28, 57-65.
- NEVILLE, D. M., JR. (1971). Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. *J. Biol. Chem.* 246, 6328-6334.
- O'FARRELL, P. H. (1975). High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250, 4007-4021.
- O'FARRELL, P. Z., and GOODMAN, H. M. (1976). Resolution of simian virus 40 proteins in whole cell extracts by two-dimensional electrophoresis: Heterogeneity of the major capsid protein. *Cell* 9, 289-298.
- O'FARRELL, P. Z., GOODMAN, H. M., and O'FARRELL, P. H. (1977). High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* 12, 1133-1142.
- OIE, M., and ICHIHASHI, Y. (1981). Characterization of vaccinia polypeptides. *Virology* 113, 263-276.
- PADHI, S. B., EIKENBERRY, E. F., and CHASE, T., JR. (1974). Electrophoresis of the proteins of the nuclear polyhedrosis virus of *Porthetria dispar*. *Intervirology* 4, 333-345.
- PHILLIPS, T. A., BLOCH, P. L., and NEIDHARDT, F. C. (1980). Protein identifications on O'Farrell two-dimensional gels: Locations of 55 additional *Escherichia coli* proteins. *J. Bacteriol.* 144, 1024-1033.
- SCOTT, H. A., YOUNG, III, S. Y., and MCMASTERS, J. A. (1971). Isolation and some properties of components of nuclear polyhedra from the cabbage looper, *Trichoplusia ni*. *J. Invert. Pathol.* 18, 177-182.
- SIDDELL, S., WEGE, H., BARTHEL, A., and TER MUELEN, V. (1981). Coronavirus JHM: Intracellular protein synthesis. *J. Gen. Virol.* 53, 145-155.
- SINGH, S. P., GUDAUSKAS, R. T., and HARPER, J. D. (1979). Serological comparison of polyhedron protein and virions from four nuclear polyhedrosis viruses of plusiine larvae (Lepidoptera: Noctuidae). *J. Invert. Pathol.* 33, 19-30.
- SINGH, S. P., GUDAUSKAS, R. T., and HARPER, J. D. (1980). Gel electrophoresis of sonicated virions of two-nuclear polyhedrosis viruses. *J. Ala. Acad. Sci.* 51, 249-255.
- SMITH, G. E., and SUMMERS, M. D. (1978). Analysis of baculovirus genomes with restriction endonucleases. *Virology* 89, 517-527.
- SMITH, G. E., and SUMMERS, M. D. (1981). Application of a novel radioimmunoassay to identify baculovirus structural proteins that share interspecies antigenic determinants. *J. Virol.* 39, 125-137.
- SUMMERS, M. D., and SMITH, G. E. (1978). Baculovirus structural polypeptides. *Virology* 84, 390-402.
- VLAK, J. M. (1979). The proteins of nonoccluded *Autographa californica* nuclear polyhedrosis virus produced in an established cell line of *Spodoptera frugiperda*. *J. Invert. Pathol.* 34, 110-118.
- WOOD, H. A. (1980). Isolation and replication of an occlusion body-deficient mutant of the *Autographa californica* nuclear polyhedrosis virus. *Virology* 15, 338-344.
- YAMAMOTO, T., and TANADA, Y. (1979). Comparative analysis of the enveloped virions of two granulosis viruses of the armyworm, *Pseudaletia unipuncta*. *Virology* 94, 71-81.
- YOUNG, S. Y., and LOVELL, J. S. (1973). Virion proteins of the *Trichoplusia ni* nuclear polyhedrosis virus. *J. Invert. Pathol.* 22, 471-472.
- ZETHNER, O., BROWN, D. A., and HARRAP, K. A. (1979). Comparative studies on the nuclear polyhedrosis viruses of *Lymantria monacha* and *L. dispar*. *J. Invert. Pathol.* 34, 178-183.