Unusual Baculovirus of the Parasitoid Wasp *Apanteles melanoscelus*: Isolation and Preliminary Characterization

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A baculovirus present in the female reproductive tract of the parasitoid wasp *Apanteles melanoscelus* has been isolated and partially characterized. Viral DNA is double stranded, circular, and of highly variable molecular weight ranging from 2×10^6 to 25×10^6 ; the DNA is of homogeneous density at $\rho = 1.694$ g/ml. Acrylamide gels resolve 18 polypeptide bands in the case of purified virions; four to five of these appear in a semipurified nucleocapsid preparation. The electrophoretic profiles obtained are compared with those of two other baculoviruses.

Enveloped particles bearing a close structural resemblance to baculoviruses are known to be present, often in considerable concentrations, in the ovaries of many parasitoid braconid wasps (27, 32, 33, 40; D. B. Stoltz and S. B. Vinson, Adv. Virus Res., in press). The "infection" is probably passed vertically, since every female wasp of any species known to harbor such particles is affected (Stoltz and Vinson, in press); no obvious detrimental effects due to the presence of these particles can be identified. Large numbers of particles are injected into caterpillars during oviposition and, at least in some cases, particle nucleocapsids subsequently interact specifically with the nuclear pores of host cells (32). It has been proposed that parasitoid particles may be involved, whether directly or indirectly, in the abrogation of host cellular immune responses to parasitoid eggs (38, 39; Stoltz and Vinson, in press). Consequently, it is hoped that some understanding of host/parasitoid interrelationships may provide new insights into the basis of immunity in insects.

On the basis of acceptable morphological criteria (32, 33; Stoltz and Vinson, in press), the braconid particles can be provisionally assigned to the baculovirus group (35, 43), which is presently being more precisely defined by the Baculovirus Study Group (International Committee on Taxonomy of Viruses). With the aim of providing confirmation of the viral nature of these particles, our laboratory has begun to examine their biochemical composition in relation to what is known about other baculoviruses. Our preliminary studies on one such particle found in the wasp *Apanteles melanoscelus* represent the subject of this report. The ultrastructure of this virus has been previously described (32; Stoltz and Vinson, in press).

(A preliminary report of this work has ap-

peared in abstract form [P. J. Krell and D. B. Stoltz, Proc. 1st Int. Colloq. Invertebr. Pathol., p. 343, 1976].)

MATERIALS AND METHODS

Rearing of parasitoid and host. Parasitoid *A. melanoscelus* (Hymenoptera: Braconidae) wasps were reared as previously described (14, 24, 32) from laboratory colonies of the white marked tussock moth *Orgyia leucostigma* (Lepidoptera: Lymantriidae); the latter were reared on an artificial diet (11, 21).

Isolation of parasitoid baculovirus. The anatomy of female braconid wasp reproductive tracts is given in Stoltz et al. (33). The portion containing virus particles (calyx and oviduct) is approximately 0.25 mm long.

Ovaries were dissected out from immobilized (0°C for 5 min) adult wasps by means of jeweller's forceps, and were separated from each other by severing the lateral oviducts with fine dissecting needles (entomological minuten pins). Fluid could subsequently be expressed into buffer solutions by applying light pressure on the calyx with dissecting instruments. Calyx fluid was routinely dissected out into small volumes (20 to 200 μ l) of Tris-EDTA buffer (10 mM Tris-1.0 mM EDTA, pH 7.5) and then subjected to low-speed centrifugation at $1,000 \times g$ for 15 min; the latter step was included to sediment any parasitoid eggs that were present. Difficulties were encountered during attempts to purify calyx particles by standard procedures (discussed in Results) so that, although further attempts to do this continue, we decided, primarily in the interests of conserving valuable material, to approach the problem indirectly, using the following rationale: if, as was known from previous experience (32), the only ultrastructurally recognizable entity in crude calyx fluid is virus, then presumably the only possible contaminants would be soluble protein and nucleic acid in the ambient fluid. It was felt that such could be removed by one or two cycles of centrifugation onto a sucrose cushion, followed by pelleting, a procedure that should result as well in maximum retention of starting material. In practice, then, the following protocol was adopted: calyx fluid from 10 or

more wasps was layered onto a discontinuous two-step sucrose gradient made up in 10 mM Tris and 1 mM EDTA (pH 7.5), consisting of a lower 1.7-ml layer of 60% sucrose and an upper 1.7-ml layer of 30% sucrose in a 4-ml IEC polyallomer tube. The sample was centrifuged at $35,000 \times g$ for 30 min at 5°C. The virus band was removed by side puncture with an 18-gauge needle and then pelleted through Tris-EDTA ($35,000 \times g$ for 30 min). This cycle was repeated, and the final pellet was resuspended and dialyzed extensively against Tris-EDTA buffer.

Purification of nucleocapsids. Calyx fluid from 40 or more wasps was expressed into 10 mM Tris-1 mM EDTA (pH 9.5) and triturated for 5 min prior to overlaying on a continuous 20 to 54% sucrose gradient made up in 10 mM Tris-1 mM EDTA, pH 9.5. Centrifugation was for 30 min at $35,000 \times g$ in an IEC SB-405 rotor. The nucleocapsid band was removed by side puncture and dialyzed against several changes of Tris-EDTA.

Purification of other baculoviruses. *Orgyia leucostigma* nuclear polyhedrosis virus polyhedra were purified from infected cadavers according to the protocol of Arif and Brown (1); virions (one nucleocapsid per envelope) and nucleocapsids were isolated and purified from polyhedra according to standard procedures (1, 3), except that 4 to 20% Ficoll gradients were used to purify virions, exactly as described by Bell and Orlob (2). Nonoccluded *Oryctes rhinoceros* baculovirus particles were purified from the midguts of infected beetles by using Ficoll gradients.

Agarose gel electrophoresis. Calyx fluid from five or more wasps was dissected out into small volumes (50 μ l) of SSC (0.15 M NaCl-0.015 M sodium citrate)-10 mM EDTA, pH 7.5, to which 2 to 4% Sarkosyl (K & K Laboratories) was then added. Samples were heated for 30 min at 60°C, after which 0.002% bromophenol blue and 15% sucrose in TEA buffer (0.05 M Tris-0.02 M sodium acetate-0.2 mM Na₂ EDTA, pH 8.5 with acetic acid; 13) were added. DNA samples were finally run in 0.7% agarose, in the same buffer, cast on a horizontal slab gel apparatus. DNA was run into gels for 30 min at 50 V, and electrophoresis was continued for another 16 to 24 h at a constant 30 V. After electrophoresis, gels were soaked in an aqueous solution of 1 μ g of ethidium bromide (EB) per ml for 1 h and then viewed on a shortwave UV transilluminator (model C-61; U.V. Products, San Gabriel, Calif.). Photography of stained gels was through a Kodak 23A filter using high-contrast copy film. Protease (Pronase or proteinase K) digestion of samples had no effect on gel profiles obtained and was therefore not used routinely. Various open circular molecular weight markers (e.g., PM2, ϕ X174 RF, and plasmids pCR1, pML21, and pSC101) were routinely included in gels, but were run in different tracks from the samples.

To confirm the presence of DNA in agarose gel profiles, enzyme digestions of gels were carried out. Four cylindrical gels, each containing material from six *A. melanoscelus* calyces, were obtained. Two gels were rinsed with 500 ml of DNase reaction buffer (0.1 M acetate buffer, pH 5, containing 0.01 M Mg₂SO₄) at 20°C for 2 h to remove EB from the stained bands. One of these remained as a control, while to the other

was added a final concentration of 0.1 mg of DNase I (Worthington) per ml. After 2 h at 20°C, both gels were again stained with 0.1% EB and examined.

The remaining two gels were washed with RNase reaction buffer (0.1 M acetate, pH 5) at 20°C for 2 h. RNase (Worthington) to a final concentration of 0.1 mg/ml was added to one. After 2 h at 20°C, gels were again stained with EB.

DNA markers. Phage ϕ X174 RF DNA was purchased from Biolabs (Beverly, Maine). PM2 DNA was a gift from A. R. Morgan of the University of Alberta. Relaxed circular plasmid markers (pCR1, pML21, and pSC101) were donated by R. Lau.

DNA purification. Calyx fluid DNA was isolated essentially by the method of Summers and Anderson (36). Calyx fluid was made to 2 to 4% Sarkosyl in SSC and heated at 60°C for 30 min. The sample was then mixed with a solution of CsCl (Calbiochem, A grade) at a density of 1.55 g/ml containing 100 μ g of EB per ml. After centrifugation in an IEC SB-405 rotor for 36 h at $130,000 \times g$ and 20°C, fluorescent DNA bands were removed by side puncture with an 18-gauge needle. DNA preparations were extracted three times with isoamyl alcohol, dialyzed extensively against SSC-EDTA, and subsequently concentrated with Aquacide. The concentrated DNA was either used directly or stored at -70°C.

In most experiments conducted to date, calyx fluid particles used for DNA isolation were twice centrifuged onto a sucrose cushion and finally pelleted. In one experiment, material thus prepared was suspended in buffer (without EDTA) containing 0.005 M MgSO₄-0.1 M acetate (pH 5) and digested with 0.008 mg of pancreatic DNase I (Calbiochem) per ml for 3 h at 25°C prior to centrifugation on a CsCl-EB gradient. Activity of the nuclease was calculated to be 56,000 U/mg by the method of Kunitz (17), using salmon sperm DNA as a substrate.

Electron microscopy. DNA, banded to equilibrium in CsCl-EB gradients, was used directly in the basic protein monolayer technique of Kleinschmidt as modified by Davis et al. (9). The spreading solution contained 20 μ l of formamide, 10 μ l of 0.2 M Tris-0.02 M EDTA buffer at pH 8.5, 10 μ l of a 0.05% solution of cytochrome *c* (Sigma, type III, aqueous), and 10 μ l of undiluted DNA. Immediately after mixing, the entire 50 μ l of DNA solution was spread onto a freshly prepared hypophase of 10% formamide in 0.01 M Tris-0.001 M EDTA (pH 8.5). Parlodion-coated grids were touched to the hypophase surface 1 min after DNA spreading. Grids were stained for 30 s in 50 μ M uranyl acetate-50 μ M HCl in 95% ethanol, dehydrated for 10 s in isopentane, and then rotary shadowed with platinum-palladium (80:20). Micrographs were taken with a Philips EM300 calibrated with a diffraction grating replica (Ladd). Circular double-stranded (ds) DNAs could not be used as internal standards with our material and were instead placed on separate grids. Micrographs of standards were taken at the beginning and end of each load of 16 plates; comparable data were obtained from many such sittings. Negatives were enlarged 10 times by projection so that the DNA molecules could be accurately traced; their contour lengths were measured with a Keuffel and Esser model 62-0300 planimeter.

Buoyant density determination. Supercoiled and open circular calyx fluid DNA was isolated from 50 wasps and extracted as described above. Each sample was mixed with *Micrococcus lysodeikticus* DNA as a buoyant density marker ($\rho = 1.731$ g/ml; 8) and centrifuged to equilibrium using a model E ultracentrifuge (22). Equivalent samples were run at three different densities of CsCl.

Polyacrylamide gel electrophoresis. Virion and nucleocapsid preparations and marker proteins (BDH, molecular weight range 14,300 to 71,500) were solubilized in 1% sodium dodecyl sulfate–1% β -mercaptoethanol in 10 mM Tris (pH 6.8) and heated at 80°C for 30 min and 100°C for 2 min. Electrophoresis in 11% acrylamide discontinuous Tris–glycine–sodium dodecyl sulfate gels was by the method of Weber and Osborn (41). After electrophoresis, gels were fixed in 12.5% trichloroacetic acid for 30 min; staining and destaining procedures were carried out at 60°C.

RESULTS

Purification of virus and nucleocapsids.

Electron microscopy reveals that the lumen of the calyx is replete with enveloped baculoviruses (Fig. 1); this calyx fluid is therefore a good source of highly concentrated virions. Light pressure on the calyx (see Materials and Methods) forces the virus-containing fluid to emerge as a thick paste, which slowly disperses into the buffer. Both Tris-EDTA and SSC-EDTA (both at pH 7.5) were found to be suitable for preserving virus morphology; Tris-EDTA was used for virus purification, and SSC-EDTA was used for DNA purification.

Initial attempts to purify the particulate component (virus) of the calyx fluid included techniques, such as rate zonal and equilibrium gradient centrifugation, now commonly employed in virus purification. However, given the amount of difficulty and time required to produce sufficient material for such experiments, standard purification protocols resulted in an unacceptable loss of material; when bands were obtained, they were very broadly smeared. We suspect at present that much of the difficulty experienced derives from the presence of the envelope protrusion (see Fig. 1), which is extremely fragile and readily sheared off, resulting in release of nucleocapsids from envelopes. Aside from that, particles are in any case heterogeneous with respect to size and number of nucleocapsids per envelope; additionally, as will be shown, nucleocapsids are themselves variable in size.

As expected, centrifugation onto a 60% sucrose cushion effectively concentrates calyx fluid particles into a thin white band. Ultrastructurally, such material contains pure virus with no obvious particulate contaminants. The sucrose cushion/pelleting protocol was subsequently used routinely to separate virus particles from

putative soluble components of the calyx fluid; the purity of the particulate fraction so obtained is discussed below in relation to both agarose and polyacrylamide electrophoresis and Kleinschmidt data.

Attempts were made to disrupt the virus membrane with Nonidet P-40 and Triton X-100 to release nucleocapsids for subsequent purification. Preliminary studies showed that the structural integrity of nucleocapsids was at least partially disrupted upon treatment with these detergents. Advantage was subsequently taken of the observation that approximately 50% of the nucleocapsids could be released from their envelopes simply by dissecting calyx fluid out in Tris-EDTA buffer at pH 9.5 (Fig. 2). Sucrose gradient centrifugation of such material concentrated nucleocapsids into a relatively broad, light-blue band at a mean density of approximately 1.13 g/ml. This band, while consisting predominantly of nucleocapsids (Fig. 3), also contained a small number of membrane fragments derived from the envelope protrusion. Nucleocapsids were found to be of constant diameter (approximately 40 nm) but variable length (28 to 100 nm). To determine if there were distinct size classes, the lengths of 186 nucleocapsids were measured and a histogram was plotted (Fig. 4). There appear to be two broad nucleocapsid length categories, one around 51 nm and the other around 66 nm. In addition, there may be several other minor length classes (e.g., at 30, 40, and 100 nm), as well as several nucleocapsids with lengths intermediate between 45 and 90 nm. It is realized that such data are subject to a degree of error, due to preparation artifact, even though negatively stained material was subject to fixation (2% buffered glutaraldehyde for 20 s) prior to air drying.

Isolation and characterization of viral DNA. Initial experiments aimed at elucidating the nature of the viral nucleic acid were carried out by means of agarose gel electrophoresis, using standard procedures for the separation and visualization of ds DNA. As expected, from the observed heterogeneity in nucleocapsid length, electrophoretic profiles of EB-stained bands were complex (Fig. 5). DNA from crude calyx fluid and from particles twice centrifuged onto a sucrose cushion reproducibly separated into about 16 bands. The presence of DNA in gel profiles was confirmed by enzyme digestion experiments (not shown): after DNase treatment no bands remained, whereas RNase digestion had no observable effect. Electrophoretic profiles of DNA from calyx particles treated with pancreatic DNase are also shown in Fig. 5. The

FIG. 1. (A) Electron micrograph of virus particles as observed in situ (*A. melanoscelsus* calyx lumen). Virions consist of several nucleocapsids enveloped by a single unit membrane. Each envelope bears a long tubular protrusion (arrow); these appendages are more readily observed in negatively stained material (B). Two nucleocapsids are encircled. Arrowheads indicate outer surface of virion envelope. Bar = 1.0 μ m. (C) Virus band resulting from sucrose gradient centrifugation. Calyx fluid in SSCE buffer (pH 7.5) was layered onto a 30 to 60% two-step sucrose gradient and centrifuged for 30 min at 35,000 $\times$ g at 5°C.

FIG. 2. Crude calyx fluid dissected into Tris-EDTA buffer at pH 9.5 and negatively stained with uranyl acetate. Compare with Fig. 1B, and note disruption of some virion envelopes (arrows) resulting in release of nucleocapsids (arrowheads). Bar = 0.7 μ m.

FIG. 3. Semipurified nucleocapsid preparation. Note variation in length of nucleocapsids and presence of "tails" at one end. Two contaminating fragments of the envelope protrusion (see Fig. 1, arrows) can be seen (double arrows). Bar = 0.5 μ m. The inset illustrates three nucleocapsids at higher magnification. Two lie in the same orientation and can be seen to differ in length (bars), while the third (on the right) is seen end-on and therefore presents a circular profile.

FIG. 4. Histogram of lengths of nucleocapsids from a nucleocapsid preparation. Electron micrograph negatives were projected onto a wall, and the end-to-end lengths (excluding the tails) were measured.

results show that, with one possible exception, there is band-for-band correspondence between crude calyx fluid, DNase-treated crude fluid, and upper-band DNA (see below) that was extracted from DNase-treated purified calyx particles. The possible exception is band 3, which appeared to be more intense in the case of DNase-treated particles. At present, we lack a satisfactory explanation for augmentation of band 3, if such occurs; conceivably, this could be due to nuclease-induced nicking of a covalently closed species (band 1?) as a result of limited leakage of enzyme into virus particles. In any case, the results strongly suggest that there are no major contaminating species of DNA in the non-particulate fraction of calyx fluid, thus confirming heterogeneity of encapsidated viral DNA. ϕ PM2 DNA, included as a control, was completely digested by the nuclease.

When calyx fluid or purified virus in SSC-EDTA was treated with 2 to 4% Sarkosyl and centrifuged to equilibrium on a CsCl-EB gradient, two fluorescent bands resulted (Fig. 6A). Crude calyx fluid from 20 wasps was sufficient for visualization of these bands; about twice as much was required in the case of virus pelleted from sucrose cushions. The upper, more intense band was at $\rho = 1.54$ g/ml and consisted predominantly of open circular DNA (Fig. 6B), whereas the lower, much less intense band was at $\rho = 1.58$ g/ml and consisted almost entirely of superhelical DNA (Fig. 6C).

As visualized by the Kleinschmidt procedure, relaxed circular DNA derived from crude calyx fluid material is highly variable in contour length (Fig. 6B). Measurements of well-spread circular molecules were made, using ϕ PM2 DNA (molecular weight, 6.4×10^6 ; 26) and ϕ X174 RF DNA (molecular weight, 3.49×10^6 ; 29) as standards. A histogram showing viral DNA contour lengths is presented in Fig. 7; ϕ PM2 DNA measurements

are also included so as to provide an estimate of contour length variability to be expected from a unique length species of DNA. The histogram reveals a major class of viral DNA contour length at 8.1 megadaltons (Mdal) and two others at 9.4 and 11.6 Mdal. In addition, six minor classes at 4.7, 6.4, 14.1, 16.3, 18.4, and 21.4 Mdal appear to be present. A small number of relatively large molecules (30 to 40 Mdal) were observed, but the majority of molecular weights were calculated to lie within the range of 2 to 25 Mdal. Contour length variability of covalently closed circular DNA has not as yet been ascertained; we would expect larger molecules to be underrepresented, due to increased chances of shearing. Whereas molecular weight data given here are for open circular DNA extracted from crude calyx fluid only, it is now clear that DNA isolated from particles that were purified and digested with nuclease (as described in Materials and Methods) is of equivalent heterogeneity (data not shown), again confirming that DNA associated with the particulate component of calyx fluid is heterogeneous.

Since in CsCl-EB gradients most of the DNA appeared in the upper band, we made the assumption that the majority of bands observed by gel electrophoresis represent relaxed circular species; accordingly, an attempt was made to see whether any correlation, in terms of molecular weight, could be made between these and the size classes identified by electron microscopy (Table 1); appropriate relaxed circular DNA markers were used in both cases to determine molecular weights (see Fig. 5 and 7; 31). As can be seen, there is fairly good correlation between size classes identified by electron microscopy and many of the major bands seen in agarose gels. Relative band positions and intensities also compare well with the histogram data. Band 9 (= peak C) appears to represent the most common molecular weight species (molecular weight = 7.9×10^6 by relative mobility or 8.1×10^6 by contour length measurement). Minor bands (1, 2, 3, 5, 7 and 8) do not appear to correspond to any of the histogram peaks; presumably, electron microscopy of a larger sample of molecules would identify additional discrete size classes corresponding to these peaks. In the absence of markers in the higher-molecular-weight range, we have not extrapolated for values above 9×10^6 , so that correlations of bands 13 to 16 with histogram peaks E to H are made on the basis of relative distribution and intensity/frequency only.

Some linear single-stranded nucleic acid was occasionally observed in upper-band material derived from crude calyx fluid, as were linear ds molecules (<20%), the latter presumably result-

FIG. 5. Electrophoresis of *A. melanoscelus* DNA in 0.7% agarose gels. (A) DNA from crude calyx fluid (four female wasps) collected in SSCE. (B) Upper band DNA isolated from a CsCl-EB gradient and dialyzed against $1 \times$ SSCE. Calyx particles from 50 female wasps were centrifuged twice onto a sucrose gradient, pelleted, and then suspended in 100 μ l of 0.1 M acetate buffer (pH 5.0) containing 5 mM MgSO_4 ; this material was then digested for 3 h at 25°C with 1 μ g of DNase I. (C) PM2 DNA (1 μ g) in 20 μ l of 0.1 M acetate buffer and 5 mM MgSO_4 . (D) PM2 DNA, as above, but digested with 0.2 μ g of DNase I for 3 h at 25°C. (E) DNA from crude calyx fluid (eight *A. melanoscelus* females) in 40 μ l of 0.1 M acetate buffer containing 5 mM MgSO_4 . (F) As in E, but digested for 3 h with 0.5 μ g of DNase I at 25°C. Track A was run approximately 1 year prior to tracks B to F (i.e., A is from a different gel). Other relaxed circular DNA markers (see the text) were subjected to electrophoresis on the same gel as track A (but on separate tracks); their relative positions and molecular weights are indicated on the left.

ing from shearing of circular forms. In a survey of 235 upper-band DNA molecules from purified, nuclease-treated particles, only 12.1% were linear ds forms.

Analytical buoyant density ultracentrifugation of either lower- or upper-band DNA in each case resolved a single peak at $\rho = 1.694$ g/ml in

cesium chloride, using *M. lysodeikticus* DNA as a marker (Fig. 8). This corresponds to a guanine plus cytosine content of 35%, as calculated according to Schildkraut et al. (30).

Proteins. Polyacrylamide gel electrophoresis of purified *A. melanoscelus* baculovirus resolved at least 18 polypeptide bands (p16 to p105; Fig.

FIG. 6. DNA from crude calyx fluid. (A) Bands appearing in CsCl-EB isopycnic gradient of DNA from calyx fluid taken from 50 *A. melanoscelus* females. The calyx fluid in SSCE was treated with 4% Sarcosyl at 60°C for 30 min, then centrifuged to equilibrium at $130,000 \times g$ at 20°C for 36 h in CsCl ($\rho = 1.58$) and EB (100 µg/ml). (B) Electron micrograph of DNA taken directly from the upper band shown in A. Note that the relaxed circular molecules vary considerably in length. Bar = 1.0 µm. (C) Supercoiled DNA from the lower band of the

FIG. 7. (A) Histogram of lengths of relaxed circular DNA molecules. Molecular weights of the different size classes were calculated using the mean size of ϕ PM2 and of ϕ X174 RF DNA as standards. (B) Distribution of lengths of circular PM2 molecules prepared in the same way and measured at the same magnification. md, Mdal.

TABLE 1. Correlation of DNA molecular weight species as determined by gel electrophoresis and electron microscopy

Agarose gel band no. ^a	Mol wt calculated from mobility in agarose gels ($\times 10^6$)	Possible corresponding peak from Kleinschmidt histogram ^b
1	—	
2	3.6	
3	4.3	
4	5.1	A
5	5.9	
6	6.1	B
7	6.6	
8	6.9	
9	7.9	C
10	8.6	
11	8.9	
12	9.1	D
13	—	
14	—	E
15	—	F
16	—	G, H

^a Agarose gel band numbers are taken from Fig. 5.

^b Peaks of DNA molecular weights are taken from Fig. 7.

9), including approximately six major polypeptides: p16, p32, p38, p51, p56, and p68. Electrophoresis of nucleocapsids resolved five: p16, p32, p38, p68, and p96, of which p32 and p38 are the major species. One or two of the minor "nucleocapsid" polypeptides could be derived from remnants of the envelope protrusion, which are found as contaminants in this material (see Fig. 3). In Fig. 9A, the staining intensity of bands p32 and p38 are roughly equivalent; often, however, the p32 band was of lesser intensity, as in nu-

cleocapsids (Fig. 9B). Particles prepared by repeated (twice or three times) centrifugation onto a sucrose cushion, followed by pelleting, exhibit a polypeptide gel profile essentially identical to that of crude calyx fluid. Thus, it does not appear that crude fluid contains any major polypeptide not associated with virions; this is not to say that minor contaminants would not be detected by methods with greater resolving power than those employed in this investigation. The molecular weights of the polypeptides were calculated

FIG. 8. Equilibrium banding of superhelical calyx fluid DNA and *M. lysodeikticus* marker DNA. DNA was taken from the lower band of a CsCl-EB gradient, EB was extracted with several changes of isopropanol, and the DNA was then dialyzed against SSCE before being mixed with marker DNA. The DNA was centrifuged to equilibrium in CsCl ($\rho = 1.7198$) at 44,777 rpm for 24 h in a Beckman model E ultracentrifuge equipped with UV optics. The position of the bands was photographed, and a scan was taken of the negative by a microdensitometer to show the relative position of the bands in the cell during centrifugation after equilibrium had been reached. Identical results were obtained with open circular DNA.

FIG. 9. Polyacrylamide gel electrophoresis of polypeptides from (A) purified *A. melanoscelus* virus; (B) semipurified nucleocapsids from *A. melanoscelus* virus; (C) purified *Oryctes baculovirus*; (D) purified *O. leucostigma* nuclear polyhedrosis virus. All samples were solubilized in 1% sodium dodecyl sulfate and 1% mercaptoethanol at 80°C for 30 min and at 100°C for 2 min before application to 11% acrylamide gels. Marker dye position (M) was marked with India ink and can be seen on the anode side of the gel. Arrowheads indicate polypeptides with mobilities equivalent to corresponding *A. melanoscelus* virus polypeptides.

from a standard curve and range from 16,500 to 105,000 daltons (Table 2).

For purposes of comparison, polypeptides of *A. melanoscelus* and two other baculoviruses, *Oryctes* virus and *O. leucostigma* nuclear polyhedrosis virus, were subjected to polyacrylamide gel electrophoresis (Fig. 9). Some similarity between the *A. melanoscelus* and *Oryctes* virus electrophoretic profiles is readily apparent, five polypeptides from both viruses having identical mobilities; of these, three are major polypeptides in both *A. melanoscelus* and *Oryctes* viruses. Homology between the polypeptide profiles of *A. melanoscelus* particles and *O. leucostigma*

nuclear polyhedrosis virus, if any, would appear to be minor, although there does appear to be some similarity in the distribution of major polypeptides: in each case, there is one major polypeptide of low molecular weight and two in the range of 32×10^3 to 44×10^3 .

DISCUSSION

In terms of morphology, baculovirus particles are enveloped cylindrical nucleocapsids that replicate in the nucleus (35); nucleocapsid dimensions are approximately 40 by 200 to 300 nm. Even in the absence of supporting biochemical information, such a description is virtually di-

TABLE 2. *Structural polypeptides of A. melanoscelus virus and nucleocapsids*^a

Virus polypeptide	Mol wt
P16 ^b	16,500
P21	21,300
P28	28,000
P30	30,500
P32 ^{b, c}	32,000
P33	33,000
P36	36,500
P38 ^{b, c}	38,000
P46	46,000
P51 ^b	51,000
P56 ^b	56,000
P65	65,000
P68 ^b	68,000
P77	77,000
P81	81,000
P87	87,000
P96	96,000
P105	105,000

^a Molecular weights of virion polypeptides were calculated with reference to the mobility of marker polypeptides in 11% acrylamide gels.

^b Major virion polypeptide.

^c Major nucleocapsid polypeptide.

agnostic for baculoviruses, since no other well-defined type of virus shares these characteristics. Also typical of baculoviruses is a genome consisting of ds circular DNA, usually of high molecular weight (70×10^6 to 90×10^6 ; 5, 12, 36). Previous investigations had suggested that on the basis of ultrastructural criteria particles observed in the ovaries of many species of braconid wasps were almost certainly baculoviruses (32, 33). In the case of *Apanteles* particles, two other points of similarity can be mentioned: (i) interaction of particle nucleocapsids with the nuclear pores of caterpillar cells (32) would appear to represent the functional equivalent of the uncoating of granulosis virus (34); and (ii) nucleocapsid tail-like appendages are morphologically indistinguishable from those associated with the nucleocapsids of *Oryctes* baculovirus (25; Stoltz and Vinson, in press).

The identification in the present study of *Apanteles* calyx particle genomes consisting of ds circular DNA (or DNAs, depending on genomic complexity) supports our previous tentative assignment of such particles to the baculovirus group (35). Nevertheless, it is clear that the *Apanteles* virus and those of other braconid wasps (27, 33) must represent an extremely unusual type of baculovirus. Thus, whereas other baculoviruses have nucleocapsids and encapsidated monomolecular genomes of uniform size, both these parameters have been shown to be extremely variable in the present instance. Extensive variability in nucleocapsid size is not

known to occur in other DNA viruses; similarly, variation in the molecular weight of encapsidated DNA molecules of other viruses also appears to be minimal (4, 42), or else involves only a small fraction of the virus particle population (e.g., 6). The only known example of a cylindrical DNA-containing virus which exhibits variation in both nucleocapsid and genome length is that of fd bacteriophage; this, however, is a single-stranded DNA virus, and genomic variation seems limited to a simple monomer/dimer relationship (42). There are, however, several examples of rod-shaped RNA viruses which exhibit nucleocapsid and genomic RNA length variation (15, 16, 18, 20), although this generally appears to be restricted to a small number (two to five) of discrete size classes. Examples of RNA virion heterogeneity are associated either with multipartite (16, 18) or defective interfering (15) systems. Which, if any, of these alternative strategies may obtain in the case of *Apanteles* particles is presently unknown.

Considerable heterogeneity in the lengths of non-encapsidated ds circular viral and plasmid DNA molecules, on a scale approaching that presented here, has occasionally been observed (19, 28, 44). What is known concerning the mode of generation of circular DNA molecules of different sizes in other such systems may provide some direction to future research on *Apanteles* viral DNAs. For example, smaller circles might represent deletion forms of larger ones, as has been shown in the case of HD and simian virus 40 papovavirus DNAs (4, 7); alternatively, larger molecules could be recombination products of smaller circular forms as suggested for the origin of simian virus 40 DNA dimers (10, 23). In any case, it seems likely that most *Apanteles* virus DNAs may share some degree of homology since purified DNA is of homogeneous density, as determined by analytical ultracentrifugation.

At least 18 polypeptide bands are resolved by polyacrylamide gel electrophoresis of solubilized, purified *Apanteles* virus. Two major nucleocapsid polypeptides with molecular weights within the range of 25×10^6 to 40×10^6 are often observed, whereas only one is seen in the case of nucleocapsids and capsids of other baculoviruses (5, 12, 37). Presumably, the main cylindrical portion of the baculovirus capsid is represented in all baculovirus gel profiles by a polypeptide of a molecular weight in the range given above. Of the two major nucleocapsid polypeptides identified, p38 seems predominant (especially in nucleocapsid gel profiles) and probably represents the capsid cylinder polypeptide. The additional major component observed in gels of *Apanteles* nucleocapsids presumably represents a putative "tail" polypeptide. It is significant, in this regard,

that a band of identical mobility (p32) also appears in polyacrylamide gels of *Oryctes* baculovirus polypeptides; as mentioned above, this virus also possesses tail-like appendages which appear to be attached to the nucleocapsids (25). Common to all three baculoviruses examined is a polypeptide of relatively low molecular weight (range 16 to 20); it is of interest that what is probably a similar polypeptide has been observed in all other baculoviruses examined to date (5, 12, 25, 37).

In toto, *Apanteles* virus polypeptides comprise a molecular weight in excess of 10^6 , which would require a minimum genome complexity of approximately 20×10^6 . This amount of information could conceivably be coded for by the larger size classes of viral DNA or by a combination of several different species; we presently favor the latter alternative. (The possibility, however remote, that some viral structural proteins could be coded for by the parasitoid genome cannot at present be ruled out; were this the case, the smallest viral DNA molecules could well code for all information contributed by the viral genome itself.) It is of interest to note that all DNA molecules purified from *Cardiochiles nigriceps* virus (31, 40) are of relatively low molecular weight (all $<10 \times 10^6$; Krell, Stoltz, and Vinson, unpublished data). Nevertheless, recent observations (unpublished) indicate that *C. nigriceps* particles contain all but two of the *A. melanoscelus* polypeptides; that is, the polypeptide composition of these two viruses is virtually identical. Since, presumably, none of the DNA molecules isolated from *C. nigriceps* particles could code for this amount (i.e., 10^6 daltons) of protein, it again seems quite possible that different molecules are coding for different polypeptides, which would require a genome complexity well in excess of 10×10^6 . We are presently examining the question of genome size in the braconid baculoviruses by means of restriction enzyme and reassociation kinetics analyses.

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