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Black Beetle Virus: Propagation in *Drosophila* Line 1 Cells and an Infection-Resistant Subline Carrying Endogenous Black Beetle Virus-Related Particles

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Black beetle virus (BBV), one of a recently discovered class of viruses with a bipartite genome, multiplied readily in Schneider's line 1 of *Drosophila* cells. Virus yields, on the order of 100 mg per liter of culture, were unusually high and represented some 20% of the total cell protein within 3 days after infection. A derivative subline of these *Drosophila* cells was found to be resistant to infection by BBV. These resistant cells were also found to carry small amounts of BBV-related particles, possibly a maturation-defective form of BBV.

Black beetle virus (BBV), originally isolated (7) from the New Zealand black beetle (*Heteronychus arator*), is characterized by an isometric 137S virion (30 nm in diameter) which contains a single major coat protein (molecular weight [mol wt], 40,000) and two RNA molecules of mol wt 1.0×10^6 (RNA 1) and 0.5×10^6 (RNA 2) (8). These properties establish BBV as a member of a recently discovered group of small divided-genome riboviruses whose prototype is Nodamura virus (10).

The unusually small genome of viruses of this group, only half as large as that of the picornaviruses or retroviruses, is especially favorable for molecular analysis. With only two RNAs, this family represents the simplest class of viruses with a segmented genome, and its members are therefore potentially useful models for studying gene interaction.

Most of our knowledge of the molecular biology of this group of viruses has been gained from studies on Nodamura virus. Translation in cell-free extracts has shown that both RNAs from Nodamura virus are active messengers (11); RNA 1 codes for a 105,000-mol-wt protein (p105) which may mediate synthesis of viral RNA, whereas RNA 2 codes for a coat-related protein of mol wt 43,000 (p43) which is probably a precursor of mature coat protein (vp40).

Progress in understanding the molecular biology of members of this interesting group of viruses requires cell culture systems suitable for propagating and manipulating virus under controlled conditions. We describe here a system involving BBV and *Drosophila* line 1 cells which allows such studies to be made. This system has led directly to the recognition of a subline of *Drosophila* line 1 which carries endogenous BBV-related particles and is resistant to infection with BBV.

MATERIALS AND METHODS

Cells. Two strains of *Drosophila* line 1 cells (15) were used. The WR strain, kindly provided by Imogene Schneider (Department of Entomology, Walter Reed Army Institute of Research, Washington, D.C.), was propagated in Schneider's complete growth medium (14) containing 5 mg of bacteriological peptone (Difco Laboratories, Detroit, Mich.) per ml and supplemented with 15% fetal bovine serum. The NZ strain was a subline of Schneider's WR strain, obtained through T. C. Grace (Canberra, Australia) and maintained for several years at Auckland, using the above growth medium but with a lower serum concentration (10%). Cells were routinely grown at 26°C in stationary tissue culture flasks (Falcon Plastics, Oxnard, Calif.; 3024) using 15 ml of medium per flask. To passage cells, confluent monolayers (5×10^7 cells) were gently dislodged by flushing with a pipette and diluted 20-fold into fresh medium.

Propagation of virus in wax moth larvae. Wax moths (*Galleria mellonella*) were raised at 30°C as described (1). Fourth- and fifth-instar larvae (12 to 15 mm long) were injected with 10 μl of a filter-sterilized BBV suspension containing about 10^6 wax moth 50% lethal doses (LD_{50})/ml (see below). An ISCO model M microinjector was used for this purpose. After 10 days at 30°C, dead larvae (typically 90 to 100% of the population) were collected and stored at −20°C.

Stock virus was prepared by homogenizing BBV-infected larvae in ice-cold 0.05 M sodium phosphate, pH 7.2, using a Sorvall Omnimixer. The 10% larval extract was clarified by centrifugation and sterilized with a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.). An equal volume of glycerol was added, and virus was stored at −20°C. Infectivity was assayed in wax moth larvae as described (1); yields averaged 10^9 to 10^{10} LD_{50} per larva.

Propagation of virus in *Drosophila* cell culture. Confluent cell monolayers were resuspended and washed in Schneider's medium containing 0.5% bovine serum albumin (BSA). Cell viability (usually greater

than 95%) was determined by staining with 0.05% Trypan blue, and the cell concentration was adjusted to 4×10^7 cells/ml. Cells (10^8) were seeded into 75-cm^2 flasks and infected with a multiplicity of 1 wax moth LD_{50}/cell, using the virus stocks described above. The infected cultures were agitated gently for 30 min and then diluted to 10^7 cells/ml with Schneider's medium containing 10% fetal bovine serum. Cultures were incubated at 26°C and frozen 4 days later at −20°C.

Purification of virus from *Drosophila* cell culture. Virus was released from infected cells with three freeze-thaw cycles. Cell debris was removed by centrifugation, and virus was pelleted through a 2.0-ml cushion of 30% sucrose in buffer A (0.05 M sodium phosphate, pH 7.2, and 0.1% β-mercaptoethanol [β-ME]) containing 0.5% BSA. Centrifugation was at 25,000 rpm in a Spinco SW25.1 rotor (60,000 × *g*) for 4 h at 6°C. Virus pellets were resuspended in buffer A. Two-milliliter samples were made 1% with respect to sodium dodecyl sulfate (SDS) and centrifuged at 25,000 rpm in the SW25.1 rotor (60,000 × *g*) for 4.5 h at 11°C on 28-ml 15 to 45% (wt/wt) sucrose gradients in buffer A. Fractions (0.6 ml) were collected from the top of each gradient, using an ISCO density gradient fractionator equipped with a flow cell (0.2-cm path length) and a model UA-5 absorbance monitor. Fractions were assayed for virus by spectrophotometric (absorbancy at 260 nm [A_{260}]) and radioactivity measurements. The virus-containing fractions were pooled, diluted with buffer A, and pelleted through a 30% sucrose cushion containing the same buffer. The virus pellets were resuspended in buffer A and stored at −70°C. Virus concentrations were calculated from optical density measurements at 260 nm, assuming that 1 mg/ml has an absorbance of 4.15 per cm of light path (8).

Preparation of radiolabeled virus. Virus propagated in *Drosophila* cell culture was radiolabeled by adding 1.0 mCi of L-[4,5-^{3}H]leucine (Amersham Corp., Arlington Heights, Ill., TRK.170) or 0.1 mCi of L-[*U*-^{14}C]leucine (Amersham, CFB.67) to each 10 ml of culture 24 h after infection. Incubation with radiolabel was continued for 3 days. Recovery of input radiolabel in purified virus ranged from 0.7 to 1.2%.

Virus propagated in wax moth larvae was radiolabeled by injecting infected larvae with 80 μCi (40 μCi/larva on days 3 and 4 postinfection) of L-[4,5-^{3}H]leucine. Virus was harvested from dead larvae 10 days after infection. About 1% of the input radioactivity was recovered in purified virus.

Electrophoretic analysis. Methods for SDS-polyacrylamide gel electrophoresis have been described by Medappa et al. (9). Gels containing 9.8% acrylamide, 0.3% (vol/vol) ethylene diacrylate, 0.1% *N,N,N',N'*-tetramethylethylenediamine, 0.1% SDS (Pierce Chemical Co., Rockford, Ill., lot 4102-5), and 1.0 M urea in 0.1 M sodium phosphate, pH 7.2, were cast in glass tubes (0.6 by 20 cm). Polymerization was catalyzed by the addition of ammonium persulfate to a final concentration of 0.05%. The electrode buffer, 0.1 M sodium phosphate, pH 7.2, containing 0.1% SDS and 0.05 M neutralized 3-mercaptopropionic acid, was circulated between upper and lower buffer vessels.

Virus samples were prepared for electrophoresis by adjusting each to 1% SDS, 0.5 M urea, 0.1% β-ME, and 10 mM sodium phosphate, pH 7.2. Samples were made 20% in sucrose and 0.01% in bromophenol blue and then heated for 5 min at 100°C before electrophoresis.

Electrophoresis was conducted at 4 V/cm until the bromophenol blue marker migrated to the bottom of the gel (about 15 h for a 20-cm gel). Gels were fractionated and analyzed as previously described (3).

Preparation of antiserum. Virus used to raise antiserum was purified from BBV-infected wax moth larvae. To purify virus, frozen larvae were homogenized in ice-cold 0.05 M sodium phosphate, pH 7.2, and carbon tetrachloride (2:1 by volume). The heavier organic phase was separated by centrifugation at 2,600 × *g*, and the virus-containing aqueous phase was clarified by centrifugation at 12,000 × *g* for 30 min. Virus was pelleted and sedimented on 15 to 45% sucrose density gradients as described above. After repelleting, virus was suspended in buffer A and stored at −70°C.

Anti-BBV serum was prepared by immunizing rabbits with a single injection of 0.5 mg of purified BBV emulsified in Freund complete adjuvant. Serum was prepared 5 weeks later.

Immunoprecipitation assays. Serological assays were conducted by the staphylococcal protein A-antibody adsorbent method described by Kessler (5). Radiolabeled virus in phosphate-buffered saline (2) containing 0.1% BSA (PBSA) was incubated with appropriate dilutions of rabbit anti-BBV serum in PBSA for 2.5 h at room temperature. Staphylococcal immunoglobulin G (IgG) adsorbent (IgGsorb; Enzyme Center, Inc., Boston, Mass.) in NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.4, and 0.02% sodium azide) containing 0.5% Nonidet P-40 and 0.1% BSA was then added to a final concentration of 1% (vol/vol). After 20 min at room temperature, the IgG adsorbent was pelleted with an Eppendorf microcentrifuge model 5412 (12,000 × *g*) and washed with NET, 0.5% Nonidet P-40, and 0.1% BSA. The adsorbent was resuspended in 0.1 M sodium phosphate, pH 7.2, containing 1% SDS and heated for 5 min at 100°C to release radiolabeled virus. The inert adsorbent was removed by microcentrifugation, and the supernatant was assayed for radioactivity.

Radioimmune competition assays. Competition assays were conducted by incubating increasing amounts of unlabeled antigen with a dilution of serum capable of precipitating 80% of a radiolabeled virus probe (0.1 μg) for 2.5 h at room temperature. A standard amount of probe (0.1 μg) was then added, and each sample was incubated for an additional 2.5 h. Virus complexed with anti-BBV IgG was precipitated by the addition of staphylococcal IgG adsorbent. The adsorbent was pelleted, washed, and assayed for radioactivity as described above.

Tryptic peptide analysis. Tryptic digestion of viral proteins and the chromatography of the resulting peptides has been described (6). Differentially labeled virus particles were mixed and heated in the presence of 0.01% SDS for 5 min at 100°C. Samples were adjusted to 0.1 M Tris-hydrochloride, pH 8.0, then reduced with 3.5 mg of dithiothreitol per ml, and alkylated with 9 mg of iodoacetamide per ml. Proteins were precipitated with 50% trichloroacetic acid and suspended in 0.1 M ammonium bicarbonate, pH 8.0. After digestion with 0.05 mg of tosylphenylala-

nylchloromethyl ketone-treated trypsin (Worthington Biochemicals Corp., Freehold, N.J.) per ml, samples were lyophilized and suspended in 0.2 M pyridine acetate, pH 3.1. Digests were filtered to remove insoluble material, and samples were chromatographed on a Chromabead type P cation-exchange column. Fractions (1.5 ml) were collected and assayed for radioactivity.

RESULTS

Preliminary studies on the propagation of BBV in cell culture. Studies on the multiplication of the small divided-genome RNA viruses have been hindered by the lack of a system in which to propagate and adequately radiolabel virus in cell culture. Although Nodamura virus grows in hamster (BHK-21) and mosquito (*Aedes albopictus*) cell culture (1), multiplication apparently occurs at such low levels that virus-specific protein synthesis has not yet been detected (11). Since the ability to radiolabel virus and virus-specific proteins in culture is crucial to studies on the molecular biology of any virus, the search for such a cell culture system has continued.

Because no plaque assay is yet available for BBV, and because the infectivity assay based on ability to kill wax moth larvae is cumbersome, the search for a susceptible line of host cells was carried out by measuring incorporation of radiolabeled uridine into virus-like particles. A number of insect and mammalian cell lines were examined. To this end, virus-inoculated cultures were incubated for 5 days in the presence of [^{3}H]uridine (50 μCi/ml). Virus, released from cells by three freeze-thaw cycles, was pelleted and sedimented on sucrose density gradients essentially as described in Materials and Methods. Virus multiplication was then monitored by examining these gradients for the presence of a 137S peak of radioactivity.

No evidence of multiplication was found in two mammalian cell lines (baby hamster kidney or mouse L-cells) nor in several insect cell lines, including those of the mosquito (*A. albopictus* and *A. aegypti*), the cabbage looper (*Trichoplusia ni*), the fall armyworm (*Spodoptera frugiperda*), or line GM1 of the fruit fly (*Drosophila melanogaster*). The virus did, however, multiply well in Schneider's *Drosophila* line 1; thus, 10 days after infection, 10-ml cultures seeded with 10^8 cells and 10^8 LD_{50} (about 10 μg) of virus yielded 4 to 8 A_{260} units, representing 1 to 2 mg of virus. Despite this high yield of virus, roughly 25 to 50 times that typically obtained from picornavirus-infected cultures, there was little cytopathic effect and virus remained largely cell associated.

Low yields of virus from a subline of *Drosophila* line 1. Preliminary attempts in Madison to grow BBV in *Drosophila* line 1 (DL-1) cells obtained from New Zealand (NZ subline) yielded only a few percent of the expected amounts of virus. The low yield was ultimately traced to the identity of the cells used to propagate the virus. Thus, low yields were obtained with the NZ subline, whereas high yields of BBV were produced by DL-1 cells obtained directly from Imogene Schneider's laboratory (WR line). This is illustrated by an experiment in which equal numbers of cells from the WR line and the NZ subline of WR cells were infected in parallel, incubated at 26°C, and radiolabeled by exposing the cultures to [^{3}H]leucine (100 μCi/ml), beginning 1 day after infection. On the 4th day after infection, virus was released by freezing and thawing and analyzed on sucrose density gradients as described above.

The parental WR cells yielded a prominent peak of radioactivity which coincided with the 137S peak of optical density (Fig. 1A). As expected, no such 137S peak was observed in the gradient from mock-infected WR cells carried in parallel (Fig. 1C). In contrast, the BBV-infected

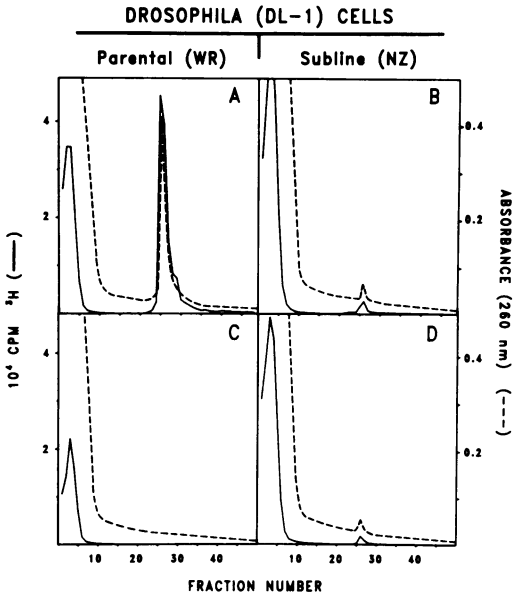

FIG. 1. *Sucrose density gradient profiles showing yields of BBV-like particles from parental Drosophila line 1 cells and its NZ subline. Parental WR cells were (A) infected with BBV and (C) mock infected. NZ subline cells were (B) infected with BBV and (D) mock infected. Cell monolayers containing 10^8 cells were exposed to 0.025 ml of 5% extracts from BBV-infected (4×10^9 LD_{50}/ml) or uninfected wax moth larvae and incubated for 4 days at 26°C. [^{3}H]leucine was added 1 day after infection. Virus was isolated, purified, and centrifuged on sucrose density gradients as described in Materials and Methods. Sedimentation is from left to right.*

NZ subline yielded only 3 to 10% as many 137S particles as the WR line (compare Fig. 1B and 1A). From optical density measurements, the total yield of virus particles from the NZ subline culture was estimated to be about 50 μg, whereas the yield from the WR parental culture was approximately 550 μg. The recovery of radioactivity in purified virus was 10^6 dpm from the NZ culture, compared with 30×10^6 dpm from the WR culture.

Unexpectedly, virus-like particles (137S) were also found in the mock-infected NZ cell cultures; moreover, they were present in amounts similar to those obtained from BBV-inoculated NZ cultures (compare Fig. 1B and 1D). The possibility that these particles arose from an undetected viral agent, inadvertently introduced by the extracts of healthy larvae used in mock infection, was ruled out in other experiments which showed the same 137S particles in NZ cells not previously exposed to larval extracts. Thus, the NZ subline of *Drosophila* cells carries endogenous particles (DL-1 particles) which sediment like BBV but which are not found in the parental WR cell line.

Electrophoretic differences in the coat protein of BBV and DL-1 particles. The similarity in sedimentation velocities suggested that DL-1 particles were related to BBV. This was confirmed by electrophoretic analysis on SDS-polyacrylamide gels. Each of the 137S peaks from the sucrose gradients shown in Fig. 1 displayed a single major band in the region of the 40,000-dalton coat protein of BBV (Fig. 2). That DL-1 particles are not, however, identical to BBV was shown by differences in their electrophoretic properties.

BBV was found to contain two coat proteins, a major one with an apparent mol wt of 40,000 (vp40), and a minor one of mol wt 44,000 (vp44) (Fig. 2); this agrees with the report of Longworth and Carey (8). vp44 may represent a precursor form of mature coat protein (vp40) as previously proposed for Nodamura virus (11); indeed, the tryptic profiles of these two BBV proteins are virtually identical (P. Friesen, unpublished data). We have observed that the relative proportion of vp44 varies from one virus preparation to another; however, it is not yet clear whether this variation reflects different contents of precursor protein vp44 in individual particles or a mixture of two different kinds of particles. DL-1 particles, unlike BBV, exhibited no evidence of a minor protein (Fig. 2C).

A second difference in the proteins of DL-1 particles and BBV was demonstrated by coelectrophoresis of differentially labeled particles (Fig. 2D). The electrophoretic mobility of DL-1 particle coat protein (mol wt 42,000) was slightly lower than that of the major BBV coat protein (vp40) but higher than that of vp44.

Finally, electrophoretic studies on the intact particles revealed a difference in net charge between BBV and DL-1 particles. At pH 8.5 (1% agarose in 35 mM Tris buffer), BBV migrated toward the cathode, whereas DL-1 particles migrated in the opposite direction (data not shown).

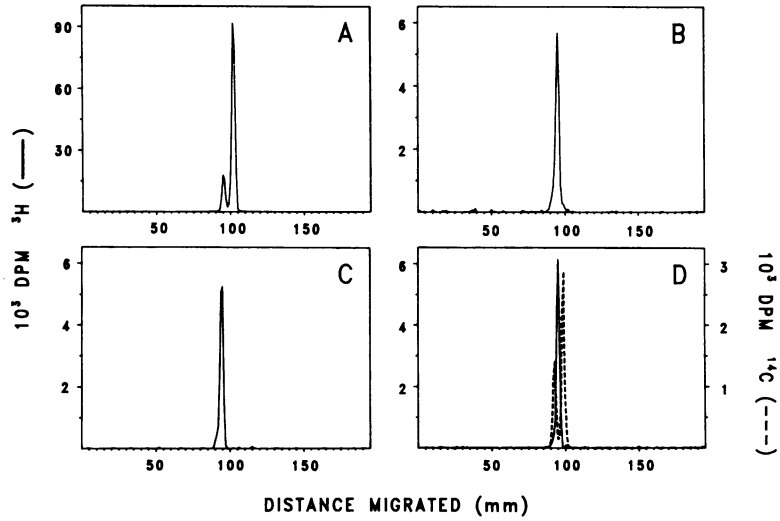

FIG. 2. *Electrophoretic profiles of coat protein of the radioleucine-labeled virus-like particles shown in Fig. 1. (A) From Fig. 1A, BBV-infected WR cells; (B) from Fig. 1B, BBV-infected NZ cells; and (C) from Fig. 1D, mock-infected NZ cells. (D) [^{3}H]leucine-labeled DL-1 particles from Fig. 1D versus [^{14}C]leucine-labeled BBV. Virus samples were disrupted and subjected to electrophoresis on SDS-polyacrylamide tube gels as described in Materials and Methods.*

Resistance of NZ cells to productive infection with BBV. Comparison of Fig. 1B and 1D shows that inoculation of the NZ subline with BBV did not significantly increase the yield of 137S material. The coat protein of particles from BBV-inoculated NZ cells was that of DL-1 particles, not BBV (Fig. 2B). The sharpness of the peak and the presence of only a slight shoulder in the region of vp40 from BBV indicates that there was little if any BBV protein in the 137S peak from BBV-inoculated NZ cells. The absence of any detectable BBV in even this small peak of virus-like material implies that NZ cells are effectively resistant to productive infection by BBV.

Serological relationship between BBV and DL-1 particles. Both BBV (derived from the WR line) and DL-1 particles (derived from the NZ subline) were precipitated by antiserum raised against BBV (Fig. 3A). The capacity of this antiserum to precipitate a constant number of particles was 12-fold greater for BBV; thus, the precipitin titer was 90,000 for BBV but only 7,000 for DL-1 particles.

Further evidence for serological relatedness was provided by a radioimmune competition assay which measured the ability of unlabeled BBV to compete with a radiolabeled DL-1 particle probe for a limited number of antibodies (Fig. 3B). The assay demonstrated that the precipitation of the DL-1 particle probe could be reduced to background levels when anti-BBV serum was preincubated with excess BBV. Thus, antibodies directed against BBV were also capable of precipitating DL-1 particles.

Gel diffusion tests using antiserum raised against DL-1 particles independently confirmed that BBV and DL-1 particles have common surface antigens (data not shown). The precipitation arcs of these tests indicated that both particles were serologically related but not identical. This nonidentity is supported by the substantial difference in precipitation titers of anti-BBV serum toward each particle (Fig. 3A).

Similarities in the tryptic fingerprints of the coat proteins of BBV and DL-1 particles. Comparison of the tryptic profiles of the coat protein of BBV and DL-1 particles, differentially labeled with radioleucine, revealed several identical tryptic fragments along with a couple of differences at fraction 114 (Fig. 4). The small number of tryptic peaks exhibited by both particles may be due in part to the insolubility of a fraction (20%) of the tryptic digest. Similar behavior has been previously observed with Nodamura virus (11) and may be characteristic of the coat protein of this class of viruses. Although the degree of difference cannot be accurately assessed because of the small number of tryptic peaks observed, the similarities among those tryptic fragments displayed again suggest that both particles are related but not identical.

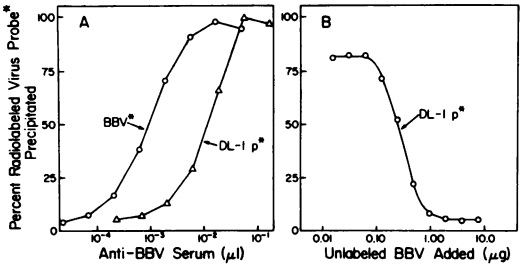

FIG. 3. *(A) Titration curves for BBV and DL-1 particles, using antiserum directed against BBV. A fixed amount (0.1 μg) of radioleucine-labeled probe antigen (BBV, ~5,000 dpm; DL-1 particles, ~1,000 dpm) in PBSA was incubated with the indicated volume of serum under standard assay conditions (2.5 h at room temperature in a total volume of 100 μl). Radioactivity in the immune complex was determined after precipitation with staphylcoccal IgG adsorbent as described in Materials and Methods. The precipitation titer, defined as the reciprocal of the volume (microliters) of serum required to half-precipitate 1 ng of virus under standard assay conditions, was 9×10^4 for BBV and 7×10^3 for DL-1 particles. (B) Radioimmune competition assay ([^{3}H]leucine DL-1 particle probe versus BBV). The indicated amounts of unlabeled BBV in PBSA were preincubated for 2.5 h at room temperature with a dilution of serum capable of precipitating 80% of the radioactive DL-1 particle probe. A standard amount (0.1 μg) of DL-1 particle probe (~1,000 dpm) in PBSA was then added, and incubation was continued under standard conditions for an additional 2.5 h. Radioactivity in the staphylococcal IgG adsorbent precipitate was determined as described.*

DISCUSSION

We have shown that BBV multiplies well in Schneider's *Drosophila* line 1. However, the infection is not very cytolytic and the infected cells remain intact for periods of 4 or more days after infection. Thus, a relatively long period of synthesis probably accounts in part for the high yields of virus which range from 1 to 2 mg per 10^8 cells (40 mg equivalent wet weight) within 3 days of infection. By the 3rd day postinfection, the cell mass has increased to about 70 mg (wet weight). Assuming that protein constitutes 10% of the cellular wet weight, 1.5 mg of virus corresponds to about 20% of the total cell-associated protein, a yield 100-fold greater than that typically obtained from picornavirus-infected cells.

Although BBV multiplies well in one strain (WR line) of Schneider's line 1, it multiplies poorly, if at all, in a derivative strain (NZ subline). The observation that the resistant NZ subline carries BBV-related particles (DL-1 par-

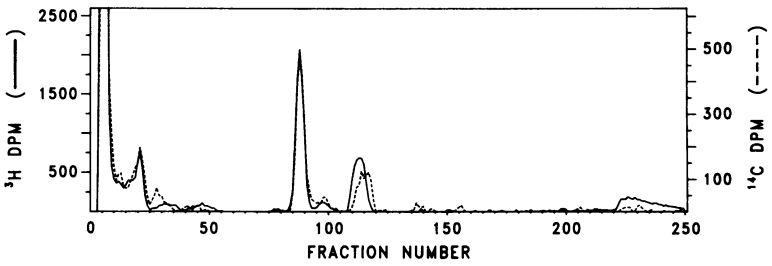

FIG. 4. *Comparison of differentially radioleucine-labeled proteins of BBV and DL-1 particles by tryptic fingerprinting. A mixture of [^{14}C]leucine-labeled BBV and [^{3}H]leucine-labeled DL-1 particles was heat denatured, digested with trypsin, and chromatographed on a cation-exchange column as described in Materials and Methods.*

ticles) whereas the susceptible WR line does not suggests that resistance to infection by BBV is mediated by the DL-1 particle. However, further evidence is required to rule out the possibility that such resistance is an independent trait of cells carrying DL-1 particles.

Recent studies (P. Scotti, unpublished data) show that DL-1 particles can multiply in cells of *Drosophila* line 2 and GM1. This infectivity of DL-1 particles not only establishes the agent as a virus (DLV), but may also open the way to clarifying the role of DLV in BBV resistance by determining its ability to alter the susceptibility of DLV-infected cells to superinfection by BBV.

The properties of DLV indicate that it belongs to the same family as BBV and Nodamura virus. Each virus sediments at 135 to 137S and contains one major coat protein in the molecular weight range of 40,000. Moreover, recent studies indicate that DLV, like BBV and Nodamura, contains two RNAs sedimenting at 15 and 22S, respectively.

The discovery of DLV raises a question about its origin. In light of reports of virus-like particles in the nucleus and cytoplasm of cultured *Drosophila* cells, including Schneider's line 1 (4, 12, 16), it is possible that DLV was a contaminant of the original tissue explant of the cell line. It could then have been either lost from the WR line or maintained in a suppressed state. Alternatively, the virus could have arisen in the NZ subline because of derepression at some later stage of subcultivation. None of these explanations, however, accounts for the remarkable coincidence that the immune *Drosophila* cell line carries an agent closely related to BBV, a virus originally isolated from black beetles.

A more likely way of explaining this coincidence is that DLV arose from an inadvertent infection of line 1 with BBV itself. Such an infection might easily have been overlooked since BBV does not cause extensive cytopathic effects in *Drosophila* cells. Continued subcultivation of the infected cells may then have selected for mutants more compatible with continued cell growth, thus leading to the establishment of a persistent infection.

The rather small differences in the tryptic profiles of the coat proteins (Fig. 4) are consistent with the hypothesis that DLV is a mutant of BBV. These tryptic similarities and the minor shift in electrophoretic mobility of the DLV coat protein suggest that relatively few changes have occurred. The apparent increase in molecular weight of the DLV protein might be accounted for by a change as small as a single amino acid residue since it has been demonstrated that replacement of such a residue can significantly alter SDS binding and thus the relative mobility of certain polypeptides on SDS-gels (see discussion in reference 6).

On the other hand, our electrophoretic and serological studies suggest that BBV and DLV differ substantially more than would be expected for a simple selection of viral mutants. For example, DLV appears to lack the minor coat found in BBV. Moreover, the particles have opposite charges at pH 8.5 and have a 12-fold difference in immune reactivity, both implying substantial differences in the surfaces of BBV and DLV.

A possible resolution to these apparently contradictory findings is suggested by an analogy with poliovirus. The surface antigenicity of poliovirus is radically altered by a large conformational change which occurs during the maturation step in which the virion acquires infectivity (reviewed in reference 13); moreover, maturation is accompanied by cleavage of each protein subunit in the precursor particle (provirion). An analogous process in BBV, whereby the protein subunits (vp44) of the provirion are cleaved to form the major coat protein (vp40) of mature virions, could also be responsible for major antigenic differences between the BBV provirion and the mature virus particle. This raises the possibility, then, that DL-1 particles represent uncleaved provirions of a maturation-defective

mutant of BBV and that the single peak of protein in DL-1 particles (Fig. 2C) represents the precursor protein, not the mature coat protein, of the defective form of BBV. It should be possible to test this hypothesis by comparing DL-1 particle coat protein with the cell-free translation product directed by its mRNA.

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