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A Single Point Mutation in the VP7 Major Core Protein of Bluetongue Virus Prevents the Formation of Core-Like Particles

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To understand the assembly process of bluetongue virus (BTV), we have established a functional assay which allows us to produce and manipulate BTV core-like particles (CLPs) composed of the viral VP7 and VP3 proteins. A cDNA clone encoding the 349-amino-acid VP7 protein has been manipulated to generate deletion, extension, and site-specific mutants. Each mutant was coexpressed with the BTV VP3 protein to generate CLPs. Deletion and extension mutants involving the VP7 carboxy terminus prevented CLP formation, while an extension mutant involving an 11-amino-acid rabies virus sequence added to the amino terminus of VP7 allowed CLP formation. Substitution of either of two cysteine residues of VP7 (Cys-15 or Cys-65) by serine also did not prevent CLP formation; however, substitution of the single lysine residue of VP7 (Lys-255) by leucine abrogated CLP formation, indicating a critical role for this lysine.

We have developed a system that allows us to investigate the interactions of different proteins that make up multicomponent structures resembling bluetongue virus (BTV) and cores (4, 5). By using multiple gene expression technologies, particles that are composed of proteins in defined but nonequimolar ratios are synthesized. These particles can be purified by gradient centrifugation, which allows their composition to be determined. The system allows a variety of studies, including analyses of the determinants of particle formation within the matrix of a cell and the effects of substitution of a component protein by alternative forms, to be undertaken.

BTV is an architecturally complex member of the *Orbivirus* genus, family *Reoviridae*, with an 810-Å (81-nm) diameter. The virus is vectored by *Culicoides* insects to certain vertebrate species, replicating in both hosts. Virions of BTV are formed from the proteins of seven viral genes (VP1 to 7) (26). These proteins are present in virions in defined but different molecular proportions. The structural proteins of BTV are organized into two shells surrounding the 10-segment, double-stranded RNA genome (22). The outer shell contains VP2 and VP5; the inner shell, or core, contains five proteins organized in three concentric structures (20). Three minor proteins (VP1, VP4, and VP6) that are probably components of the viral RNA-directed RNA polymerase and associated enzymes are associated with the double-stranded RNA genome and form the innermost part of the core. This nucleocapsid is surrounded by a layer of the 100-kDa VP3 protein, forming the subcore. On top of VP3 is located a layer of the 38-kDa VP7 protein, forming the core.

Recent data obtained by cryoelectron microscopy (to 30-Å [3-nm] resolution) of unfixed and unstained cores derived from BTV indicate that there are several unique features in the structure of the 690-Å (69-nm) core (20). The core has a bristly appearance and icosahedral symmetry exhibiting a triangulation number of 13. The appearance is due to 260 prominent knob- or spike-like structures at all the local and strict threefold axes. The spikes have been shown by both biochemical and crystallographic analyses to be trimers of VP7 (1, 6, 20). The trimers are connected at a lower radius to a smooth layer of VP3 protein (17, 20).

Using multiple gene expression vectors, we have coexpressed the VP3 and VP7 proteins of BTV and obtained empty core-like particles (CLPs). In terms of their sizes, 250S values, electron microscopy appearance, and stoichiometries of the two proteins, CLPs are similar to cores derived from infectious BTV (5). Cryoelectron micrographs of CLPs not only revealed an icosahedral structure and knobs of VP7 trimers similar to those of virion-derived cores but also showed that the smooth inner shell of CLPs is composed of 60 copies of VP3, arranged in 12 pentamers (6). VP7 trimers have been removed from CLPs by dialysis, which left a smooth-surface subcore composed only of VP3 (17). However, subcores composed of VP3 apparently do not assemble in vivo in the absence of VP7, nor do BTV VP7 trimers form any morphological entities without VP3 (17), indicating a critical role of VP7 in the genesis of CLPs.

We have recently reported data which indicate that the two outer capsid proteins of BTV, VP2 and VP5, attach to the core, most likely involving VP7 (7, 15). Because of its multifarious roles, VP7 offers an interesting model to experimentally define intermolecular interactions in the BTV assembly process. Towards this end, we have initiated an investigation of the domains of VP7 that are involved in particle assembly.

In this report, we generated a number of site-specific mutants and deletion or extension mutants of VP7 and expressed them in the presence of VP3 to generate CLPs using multiple gene expression vectors based on the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV). The data indicate that the intact carboxy terminus of the VP7 molecule is essential for particle formation but that the amino terminus can be modified by the addition of

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some foreign sequences without compromising the ability to form CLPs. Replacement of the single lysine residue of VP7 with leucine abrogated CLP formation, although replacement of either of the first two conserved cysteine residues (Cys-15 or Cys-65) (11, 12, 21) with serine did not prevent CLP formation.

MATERIALS AND METHODS

Virus and cells. *Spodoptera frugiperda* (*Sf*) cells were grown in suspension or monolayer cultures at 28°C in TC100 medium supplemented with 10% fetal calf serum. AcNPV and recombinant derivatives were plaque purified and propagated (2).

Deletion mutation of the BTV VP7 gene and construction of recombinant transfer vectors. For the deletion construct, the C-terminal 14 amino acid residues (336 to 349) of the BTV serotype 10 (BTV-10) VP7 gene were deleted by using the polymerase chain reaction. The plasmid pAcYM1.BTV-10.7 (19), containing the complete coding sequence of the BTV-10 VP7 gene, was used as a template in the polymerase chain reaction. The forward-sense primer 5′ GGCCGGATCCG TCG-ACTATAGAGATG 3′ and the reverse-sense primer 5′ GGCCGGATCCCTACATCGG-ATTTACGCC 3′ were used to introduce *Bam*HI sites at both ends (underlined) of the VP7 sequence as well as to insert a TAG stop codon (double-underlined complementary reverse sequence CTA) immediately following the VP7 methionine at position 335. The amplified product was repaired with the Klenow large fragment of DNA polymerase I, purified by GeneClean (Promega) as described by the manufacturer, and inserted into pUC118 that had been previously digested with *Sma*I and dephosphorylated. Recombinant plasmids were isolated and identified by restriction enzyme mapping, and the sequence at the insertion site was confirmed by the dideoxynucleotide technique (23). The full-length insert was excised by digestion with *Bam*HI and ligated to the *Bam*HI site of pAcYM1 baculovirus transfer vector (18). A recombinant plasmid was designated pAcYM1.BTV-10.7 CT.

Site-directed mutagenesis, insertion of additional sequences in VP7, and the construction of recombinant transfer vectors. Site-directed mutagenesis was used to mutate each of the two conserved cysteines in the BTV-10 VP7 gene (Cys-15 and Cys-65 [see Fig. 1]) to serine residues (i.e., TGT to TCT). Similarly, the unique lysine residue at position 255 (Lys-255) in the BTV-10 VP7 sequence was converted to a leucine residue (i.e., AAA to TTA). To facilitate the preparation of these VP7 mutants in the baculovirus system, the BTV-10 VP7 gene was initially subcloned into pAcCL29, a pAcYM1-based baculovirus transfer vector with single-stranded capability (16). With appropriate oligonucleotides, the indicated sequences were mutated, and recombinant plasmids (pAcCL29.BTV-10.7.C_{15}, pAcCL29.BTV-10.7.C_{65}, and pAcCL29.-BTV-10.7.K_{255}) were identified and verified by sequence analyses (23).

To introduce a rabies virus-specific epitope into the amino or carboxy end sequence of VP7, the gene was initially mutated to provide unique restriction sites at both ends of the gene in order to allow insertion of complementary oligonucleotides with 5′ and 3′ overhangs and provide the rabies virus epitope KSVRTWNEIIP (25) either immediately before the VP7 carboxy-terminal stop codon or, with an additional methionine residue in front of the rabies virus sequence, in lieu of the VP7 initiating ATG codon. The inserted sequences in the resulting recombinants, pAcYM1.BTV.VP7 C-E and pAcYM1.BTV.VP7 N-E, were verified by sequence analyses.

Isolation of recombinant baculoviruses. Recombinant baculoviruses were generated by cotransfection of the respective recombinant transfer vector DNAs with linearized AcRP23.LacZ baculovirus DNA by using the Lipofectin technique (10). Recombinant viruses (VP7 CT, VP7 Cys_{15}, VP7 Cys_{65}, VP7 Lys_{255}, VP7 C-E, and VP7 N-E) were initially identified on the basis of their white-plaque phenotype (after staining with X-Gal [5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside]). To confirm that the altered BTV-10 VP7 proteins were expressed from the different constructs, extracts of *Sf* cells infected with each recombinant baculovirus were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% polyacrylamide) as well as by Western blotting (immunoblotting) with an anti-BTV-10 polyclonal serum as described previously (13).

Purification of CLPs. *Sf* cells were coinfected at multiplicities of 5 PFU of each virus with the indicated VP7 (AcBTV-10.7) or mutant (VP7 CT, VP7 C-E, VP7 N-E, VP7 Cys_{15}, VP7 Cys_{65}, or VP7 Lys_{255}) baculovirus and AcBTV-17.3 (8). Infected cells were harvested at 36 to 48 h postinfection, washed with phosphate-buffered saline, and lysed at 4°C in 50 mM Tris HCl [pH 8.0]–150 mM NaCl–0.5% Nonidet P-40. Following centrifugation at 1,500 × *g* for 10 min, the cytoplasmic supernatant was either loaded onto a discontinuous sucrose gradient (30 to 66% [wt/vol]) in 0.2 M Tris HCl, pH 8.0, and centrifuged at 85,000 × *g* for 3 h at 4°C or purified by CsCl gradient centrifugation as described previously (5). Noncentrifuged samples were used as controls to monitor the expression of the VP3 and VP7 proteins by SDS-PAGE. The recovered CLPs were analyzed by SDS–10% PAGE or Western analysis or examined by electron microscopy.

Electron microscopy. Formvar carbon-coated grids were floated for 2 min on droplets of the sample recovered from the gradients. After being washed twice in 0.2 M Tris HCl, pH 8.0, the material was stained for 20 s on droplets of 2% uranyl acetate. All grids were examined with a JEOL electron microscope.

RESULTS

The carboxy terminus of VP7 is essential for core formation. The carboxy terminus of VP7 was investigated for its role in CLP formation by deletion and in-frame addition of sequences (Fig. 1). An 11-amino-acid, immunogenic epitope representing rabies virus glycoprotein (G protein) was added to the carboxy terminus of the BTV-10 VP7 gene and the chimeric protein (VP7 C-E) expressed by a recombinant baculovirus. The expression of the modified protein was identified by SDS–10% PAGE of extracts of infected insect cells. By comparison with wild-type AcNPV or a recombinant that expresses the unmodified 38-kDa VP7 protein, a protein of slightly larger size was identified in stained extracts of infected cells (data not shown). Like unmodified VP7 (Fig. 2A, lane 1), the larger VP7 was recognized by anti-BTV-10 serum (Fig. 2A, lane 2), confirming that it was related to VP7. The protein was also recognized by a rabies virus polyclonal antiserum as well as by a monoclonal antiserum (50ADI) specific to the rabies virus G protein (data not shown) (21). As a positive control for the rabies virus antiserum, baculovirus-expressed rabies virus glycoprotein (G; Fig. 2B, lane 1) was used (21). To determine whether the chimeric VP7 would assemble with the BTV VP3 protein and form CLPs, a suspension culture of *Sf* cells was coinfected

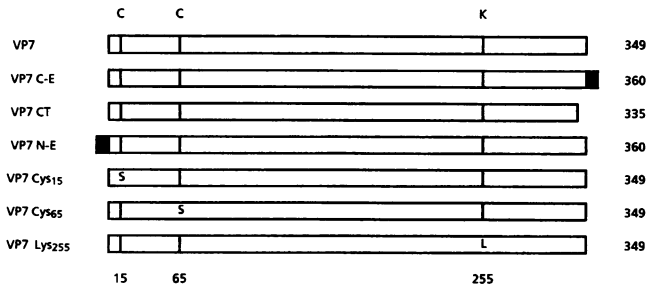

FIG. 1. Diagram of recombinant baculoviruses expressing various VP7 mutants, showing schematics of the various VP7 mutants employed in this study and their sizes (numbers of amino acids) compared with the 349-amino-acid VP7 protein. The positions of two conserved cysteine residues (Cys-15 and Cys-65) and the single lysine residue (Lys-255) of VP7 and their substituents (serine [S] for cysteine and leucine [L] for lysine) are shown. The positions of the carboxy- and amino-terminal extensions (VP7 C-E and VP7 N-E, respectively) and carboxy-terminal truncation (VP7 CT) are indicated.

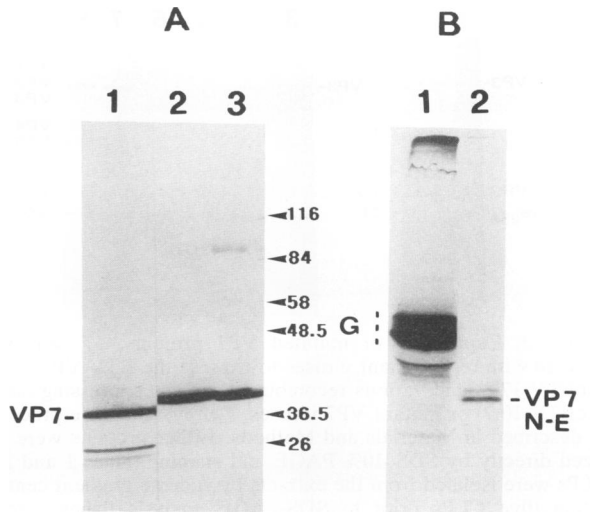

FIG. 2. Western blot (immunoblot) and immunoprecipitation analyses of carboxy and amino terminus extension mutants of VP7. *Sf* cells were infected with recombinant baculoviruses containing the BTV VP7 gene (AcBTV-10.7) or a modified VP7 gene with either a carboxy- (VP7 C-E) or an amino-terminal extension (VP7 N-E). Cell extracts were resolved by SDS–10% PAGE and then subjected to Western analysis or immunoprecipitation. (A) BTV-10 antibody was employed in the Western analyses to identify VP7. Lane 1, unmodified VP7; lane 2, the carboxy-terminal extension mutant; lane 3, the amino-terminal extension mutant). Molecular sizes in kilodaltons are shown on the right. (B) Rabies virus polyclonal antiserum was employed in the immunoprecipitation analyses of lysates of *Sf* cells infected with a recombinant virus expressing the variously glycosylated forms of rabies virus glycoprotein (G) (lane 1) (21) or one expressing the VP7 N-E protein (lane 2).

with the recombinant virus and another recombinant that expresses VP3 (AcBTV-17.3) (8). Both proteins were identified in extracts of infected cells by SDS-PAGE (data not shown). However, no morphological structures in thin sections of infected cells were observed by electron microscopy (data not shown). Also, no visible band of CLPs was detected following sucrose or cesium gradient centrifugation of cell extracts (5).

The inability of the VP7 protein modified at the carboxy terminus to be incorporated into CLPs when coexpressed with VP3 suggests an essential structural role of this terminal region of VP7 protein for assembly into the BTV core particle. To further investigate this question, an expression vector with a truncated VP7 protein lacking residues 336 to 349 at the carboxy terminus (VP7 CT) (Fig. 1) was produced and expressed in the presence of VP3 (Fig. 3, lane 2). By comparison to the unmodified VP7 and VP3 proteins that formed CLPs (Fig. 3, lane 7), no CLPs were recovered following sucrose gradient centrifugation of cell extracts (Fig. 3, lane 5). Also, no evidence for CLP formation in thin sections of infected *Sf* cells analyzed by electron microscopy was obtained (data not shown). Whether the truncated VP7 was incapable of oligomerization and thereby unable to form CLPs or whether the carboxy-terminal residues are directly involved in interaction with VP3 protein remains to be investigated. Interestingly, the hydropathic plot of VP7 protein indicates the existence of hydrophobic domains involving the last 50 amino acid residues of VP7. Such domains may be involved in intra- or intermolecular interactions important for the function of the protein.

The amino terminus of VP7 can tolerate additions when involved in the formation of CLPs. The amino terminus of VP7 is generally hydrophilic (19, 27). To investigate whether the amino terminus of the protein can tolerate additional sequences, an extension mutant with an additional 11-amino-acid rabies virus sequence (VP7 N-E [Fig. 1]) was prepared. The chimeric gene was expressed by a recombinant baculovirus in the presence of unmodified VP3. As shown in Fig. 2A (lane 3), by comparison with native VP7 protein (lane 1) of 38 kDa, a larger VP7 protein was identified by Western blot analysis. The faster migrating band in the control lane (Fig. 2A, lane 1) is probably a degraded product of the VP7 protein. The VP7 protein was recognized by rabies virus antibody, confirming that the protein possesses the rabies virus G protein epitope (Fig. 2B, lane 2). To investigate whether the recombinant protein would assemble with the BTV VP3 protein, *Sf* cells were coinfected with AcBTV-17.3 and VP7 N-E, and the infected cells were harvested, lysed, and analyzed by sucrose or CsCl gradient centrifugation as described previously (5). Particles were detected in the same position as CLPs composed of VP3 and unmodified VP7. When the protein components were analyzed by SDS-PAGE, both VP3 and the chimeric VP7 were identified in cell extracts (Fig. 4, lane 5) and in the derived particles recovered following sucrose gradient centrifugation (Fig. 4; compare lane 11 with lane 10 [CLPs obtained from VP3 and unmodified VP7]). As expected, the particles with the modified VP7 reacted with an anti-rabies virus antiserum (Fig. 4, lane 12), indicating the presence of the rabies virus epitope. Interestingly, when the same preparation was resolved by CsCl gradient centrifugation, the particles lacked most of the VP7 component (Fig. 4, lane 7). However, when particles were produced from coinfections with AcBTV-17.3, AcBTV-10.7, and VP7 N-E, both the modified and the unmodified versions of VP7 were recovered in CLPs following cesium chloride gradient centrifugation (Fig. 4, lane 8). Both VP7 proteins were also identified in CLPs following sucrose gradient centrifugation of the same preparation (data not shown). The results suggest that the modified VP7 may attach to subcores composed of VP3 but that the attachment is sensitive to removal under high-salt conditions as with CsCl, except when authentic VP7 is present. In the latter case, the unmodified VP7 appears to stabilize the association of the modified form.

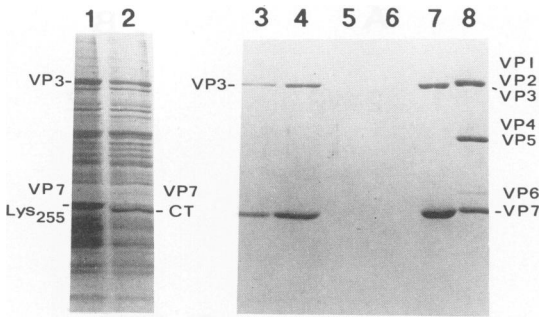

FIG. 3. Expression of mutated VP7 proteins. *Sf* cells were infected with recombinant viruses to express the BTV VP3 protein (AcBTV-17.3) and various recombinant viruses expressing normal (AcBTV-10.7) or mutant VP7 viruses. Cell lysates were processed as described in Materials and Methods. Either proteins were analyzed directly by SDS–10% PAGE and staining (lanes 1 and 2) or CLPs were isolated from the extracts by sucrose gradient centrifugation (five CLPs prior to SDS-PAGE analysis (lanes 3 to 8). Lysates of cells infected with AcBTV-17.3 and VP7 Lys_{255} (lane 1) and cells infected with AcBTV-17.3 and VP7 CT (lane 2) are shown. CLPs formed by VP3 and recombinants that expressed VP7 Cys_{15} (lane 3), VP7 Cys_{65} (lane 4), VP7 CT (lane 5), VP7 Lys_{255} (lane 6), or unmodified VP7 (AcBTV-10.7) (lane 7) are also shown. Lane 8, profile of BTV proteins (the minor protein bands, VP1, VP4, and VP6, were only barely visible).

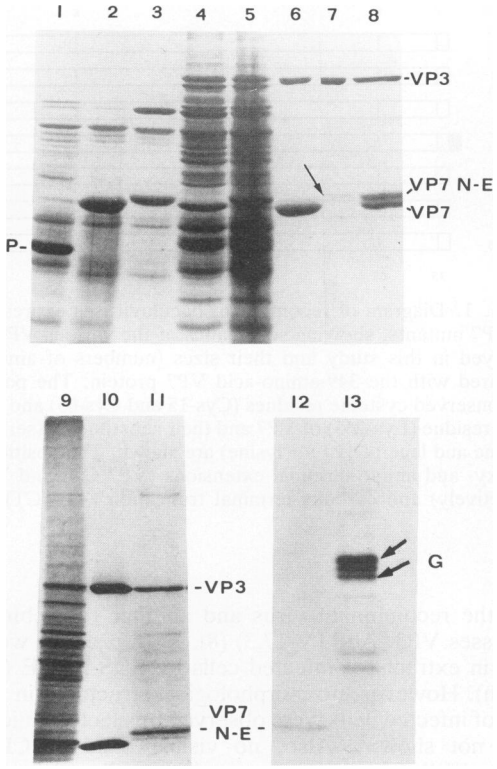

FIG. 4. Analysis of VP7 modified at the amino terminus with a rabies virus G protein sequence. *Sf* cells were infected with AcNPV (lane 1), AcBTV-10.7 to express the unmodified VP7 protein (lane 2), or VP7 N-E to express a VP7 amino-terminal fusion involving a rabies virus epitope (lane 3). Alternatively, *Sf* cells were coinfected with AcBTV-17.3 and AcBTV-10.7 to express both VP3 and unmodified VP7 (lane 4) or AcBTV-17.3 and VP7 N-E to express VP3 and the mutant VP7 (lanes 5 and 9), and extracts were resolved by SDS-PAGE and then stained. CLPs purified by cesium chloride gradient centrifugation for VP3 and unmodified VP7 (lane 6), AcBTV-17.3 and VP7 N-E (lane 7), and AcBTV-17.3, AcBTV-10.7, and VP7 N-E (lane 8) are shown. The faint band of modified VP7 in lane 7 is indicated by an arrow. CLPs purified by sucrose gradient centrifugation for VP3 and unmodified VP7 (lane 10) and for VP3 and the VP7 N-E protein (lane 11) are also shown. Lane 12, immunoprecipitation results of the VP3–VP7 N-E CLPs obtained with an anti-rabies virus serum, compared with the results of immunoprecipitation of *Sf* cell extracts containing baculovirus-expressed G protein (lane 13) (21). The positions of the AcNPV polyhedron (P), the variously glycosylated forms of the rabies G protein (arrows), and BTV VP3, VP7, and modified VP7 N-E proteins are indicated.

Shown in Fig. 5A are electron micrographs of CLPs derived from VP3 and VP7 N-E isolated by sucrose gradient centrifugation. They revealed morphological structures similar to authentic CLPs (5). By contrast, the particles isolated from the same preparation following CsCl centrifugation (Fig. 5B) lacked much of their surface structure and resembled VP3 subcores that can be derived from CLPs composed of VP3 and VP7 (17). In addition, storage at 4°C of CLPs composed of VP7 N-E and VP3 that had previously been purified by sucrose gradient centrifugation led to the loss of the surface capsid structures and derivation of subcore particles similar to those shown in Fig. 5B. By contrast, the structures made with both mutant and normal forms of VP7 were stable upon storage at 4°C in 0.2 M Tris, pH 8.0, for periods of >6 weeks. Taken together, the data indicate that although the addition of this foreign sequence at the N terminus of VP7 did not prevent CLP formation, the interaction was not strong enough to be stable under high-salt or prolonged-storage conditions without the presence of unmodified VP7.

The first two cysteine residues (Cys-15 and Cys-65) of VP7 are not required for the formation of CLPs. The VP7 protein of BTV-10 contains 349 amino acids. The protein sequence is highly conserved among various BTV serotypes (14, 19). It also has strong homologies with the corresponding proteins of African horse sickness virus (AHSV) (23) and epizootic hemorrhagic disease virus (EHDV) (9). In these viruses, the region with the greatest concentration of conserved residues is the first 100 amino acids at the N terminus (9). Within this region, there are two cysteine residues (Cys-15 and Cys-65) which are shared by all three viruses. BTV-10 VP7 contains one other cysteine residue (at amino acid residue 154), while AHSV serotype 4 contains three cysteines (residues 161, 195, and 273). It is possible that the two conserved cysteine residues (Cys-15 and Cys-65) may be important for the structural integrity of VP7 proteins, possibly involving intramolecular disulfide linkages. To investigate whether the cysteines affect the function of VP7 as measured by its ability to form CLPs, each cysteine was mutated to a serine residue (Fig. 1) and the two altered genes were expressed by using baculovirus vectors. The expressed proteins were examined by SDS-PAGE, and their antigenic authenticities were confirmed by Western analyses using anti-BTV-10 antisera (data not shown). Subsequently, the abilities of the mutant proteins to interact with VP3 protein and to form CLPs were determined by coinfecting *Sf* cells with AcBTV-17.3 and a vector containing a mutant virus (VP7 Cys_{15} or VP7 Cys_{65}). The assembled particles were isolated by sucrose gradient centrifugation, and their protein components were analyzed by SDS-PAGE. As shown in Fig. 3, both mutants (lanes 3 and 4) formed CLPs similar to normal CLPs

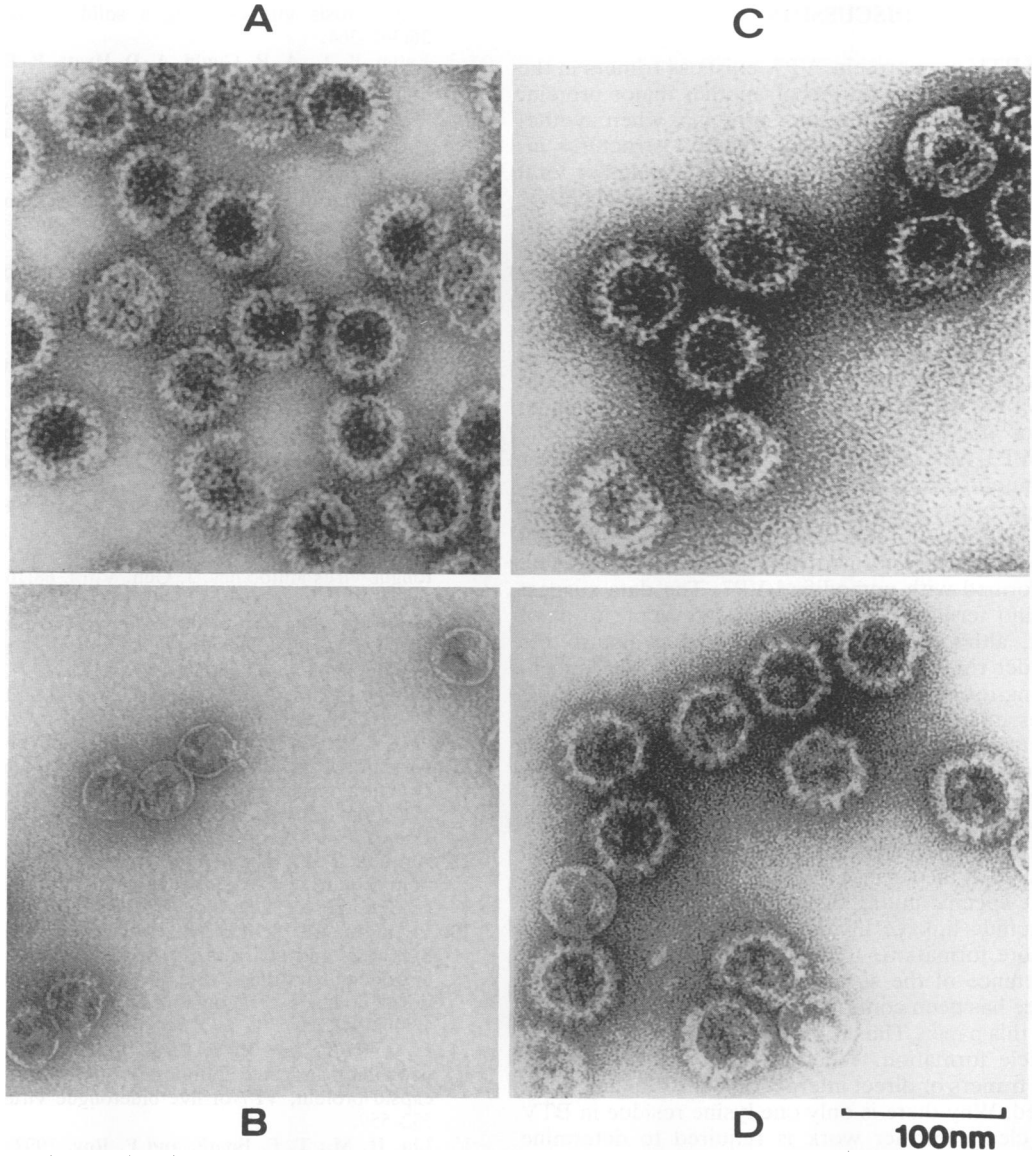

FIG. 5. Electron micrographs of CLPs produced from VP3 and mutant VP7 species. (A) CLPs prepared from VP3 and VP7 N-E and purified by sucrose gradient centrifugation; (B) the same preparation purified by CsCl gradient centrifugation, with much of the outer VP7 N-E protein absent. (C and D) CLPs produced from VP3 and one of the cysteine mutants, VP7 Cys_{15} or VP7 Cys_{65}, respectively.

(lane 7) and contained essentially similar ratios of VP3 and VP7. The morphologies of the particles were analyzed by electron microscopy (Fig. 5C and D). CLPs formed with the two mutant VP7 proteins were similar in appearance to normal VP3-VP7-derived CLPs. Moreover, like authentic BTV cores and CLPs, they were physically stable, as indicated by storage of the particles at 4°C in 0.2 M Tris, pH 8.0, for >6 weeks.

The single lysine residue at position 255 of the BTV-10 VP7 protein is required for CLP formation. The VP7 sequence of all BTV serotypes analyzed thus far contains only one lysine residue (amino acid residue 255) (12, 27). The aligned EHDV VP7 sequence also has a lysine at this position, while the aligned AHSV VP7 protein has an arginine at this position (19). All three viruses have arginine residues, some of which are conserved while others are not, at other sites (9). In addition, EHDV has only two other lysines (residues 13 and 268) (9), while AHSV has a single lysine at residue 120 (23). The general paucity of lysines and the presence of lysine at residue 255 in the BTV and aligned EHDV sequences (and arginine in the AHSV sequence at that position) suggests that this lysine may have an important structural role.

In order to investigate the role of this lysine, a mutant VP7 gene in which the lysine was substituted by leucine was prepared. As shown in Fig. 3 (lane 1), the protein was expressed to high level but did not form CLPs, as evidenced by electron microscopy of infected cells (data not shown) and the lack of recovery of CLPs following sucrose gradient centrifugation of infected *Sf* cell extracts (Fig. 3, lane 6). The results indicate that the BTV lysine at residue 255 either directly or indirectly influences the interaction of VP7 protein with VP3 in the formation of CLPs. Either VP7 does not fold correctly to form trimers or the site of interaction between VP7 and VP3 requires this residue.

DISCUSSION

The major BTV core protein, VP7, exists as trimers in the BTV virion. It is located on top of another major protein, VP3, in the virion core. These two proteins, when synthesized simultaneously, assemble into corelike structures independent of the presence of other virion proteins or viral double-stranded RNA. On the basis of this knowledge, we have established a functional assay for identifying regions of VP7 necessary for CLP formation. Our studies involved generating deletion and extension mutants of VP7 that were subsequently expressed by recombinant baculoviruses in the presence of VP3. The data have documented that the C terminus of VP7 is required for CLP formation, possibly because of a need to establish intermolecular interactions in the structure. When carboxy-terminal mutants (containing extensions or deletions) of VP7 were expressed in the presence of VP3, no CLPs were formed. In contrast, similar extension mutants, having 11 extra amino acid residues representing a rabies virus G protein epitope at the amino terminus of VP7, did not prevent CLP formation, although the structures that were formed were physically less stable than those formed with unmodified VP7. The data suggest that the amino terminus can accommodate some form of modification, although this modification may disturb the structure under rigorous conditions (e.g., high salt concentration or long-term storage). The fact that when VP7 N-E was coexpressed with unmodified VP7 and VP3 stable CLPs were formed suggests that some form of a hybrid trimer structure that is more stable than structures made of mutant VP7 alone may be made. The data support the recent report of Eaton and associates (3), who suggested that an amino-terminal region of VP7, near the cysteines at positions 15 and 65, is accessible at the surface of the BTV core. Our studies involving site-specific mutagenesis in each cysteine suggest that any disulfide linkage involving these cysteines is not critical for core formation.

The importance of the single lysine residue in the BTV VP7 sequence has been confirmed by the mutagenesis result described in this paper. The data suggest that it has a critical role in particle formation. Whether this role involves the formation of trimers or direct interaction with VP3 has yet to be established. Why there is only one lysine residue in BTV VP7 is not clear. Further work is required to determine whether the lysine can be substituted with arginine or histidine and still maintain an ability to form cores. In this context, it is noteworthy that AHSV has an arginine residue in a position comparable to that of the lysine in the BTV VP7 sequence. X-ray diffraction analyses of VP7 that are under way (1) should shed some light on the structural importance of the BTV VP7 lysine.

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