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Expression of the Major Capsid Protein VP6 of Group C Rotavirus and Synthesis of Chimeric Single-Shelled Particles by Using Recombinant Baculoviruses

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VP6 of group C (Cowden strain) rotavirus was expressed in the baculovirus system. The recombinant protein, expressed to a high level in insect cells, was purified by ion-exchange chromatography. The purified protein was proven to be trimeric. The effect of pH on the trimer's stability was investigated. Coexpression of VP6 from group A (bovine strain RF) and VP6 from group C in the baculovirus system did not result in the formation of chimeric trimers. Coexpression of VP2 from group A rotavirus (bovine strain RF) and VP6 from group C in the baculovirus system led to the formation of chimeric, empty, single-shelled particles. These results demonstrate conservation in the domains necessary for binding to VP2 in different serogroups of VP6. The locations of the domains involved in trimerization and in the interaction with VP2 are discussed.

Rotaviruses, members of the *Reoviridae* family, have a segmented double-stranded RNA genome enclosed in a three-layered protein capsid (11, 13, 33). The most external layer of proteins consists of VP4 and VP7. It is removed in vitro by calcium chelation and very probably in vivo in the cytoplasm of the infected cell. The inner capsid is formed by VP6. Treatment of single-shelled particles with chaotropic agents leads to the formation of core particles (1), consisting of VP2, VP1, VP3, and genomic RNA. The last two proteins present significant similarities with other RNA-dependent RNA polymerases (9, 25, 30) and are probably involved in genome transcription (44).

VP6 is the major outer protein of the single-shelled particles (1, 12) and represents 50 to 60% of the total mass of group A virions (26). It has been shown that VP6 assembles in trimers and tubules (12, 16, 34, 38). It has been suggested, however, that VP6 is a tetramer in the viral capsid of group C rotaviruses (20). VP6 is also implicated in the endogenous transcriptase, since removal of the inner capsid results in the loss of transcriptase activity, which is restored when VP6 is reassociated with the particles (1, 40, 41). Conversely, VP6 is not present in the subviral particles that express the replicase activity of the virus (29).

VP6 is highly immunogenic and antigenic and is the most frequently detected protein in diagnostic and immunological assays. It bears group and subgroup epitopes (17, 43). The VP6 of a group A virus (SA11 strain) has been expressed in the baculovirus system (12). Coexpression of VP2 (RF strain) and VP6 (SA11 strain) by insect cells resulted in the formation of empty single-shelled particles called pseudo-single-shelled particles (22). The flexibility of the insect-baculovirus expression vectors allows this system to be used for the expression of multiple genes and for investigation of the molecular interactions of viral proteins (15, 27).

Group A and group C rotaviruses have no common antigen, and until now, reassortants between group A and group C rotaviruses could not be isolated in vitro. Infections caused by group C rotaviruses have been described for humans, pigs, and bovines (19). Most of the human cases have been described as sporadic (4), but recently, outbreaks have been observed (5), one of which proved fatal (6). Though the Cowden strain of group C rotavirus has already been adapted to cell culture (39), this virus grows poorly. The absence of immunological reagents has made it difficult to evaluate the importance of the non-group A rotaviruses as infectious agents in animals and humans. Obtaining the major antigen of group C viruses would help to establish diagnostic tests.

A full-length cDNA clone (2) of segment 5 for rotavirus group C (Cowden strain) was expressed to high levels in the baculovirus system. In this article, we describe the production of chimeric single-shelled particles by coinfection of insect cells with two recombinant baculoviruses, one expressing group A VP2 (VP2A) and one expressing group C VP6 (VP6C). The characteristics of oligomerization of VP6 were determined by using the recombinant protein.

MATERIALS AND METHODS

Virus and cells. Wild-type and recombinants of *Autographa californica* nuclear polyhedrosis virus (AcNPV) were grown in monolayers of *Spodoptera frugiperda* 9 (Sf9) cells as described previously (42).

Construction of recombinant baculoviruses. The full-length cDNA clone of the Cowden strain gene 5 (2) was digested with *Dra*I and subcloned into the transfer vector pVL941 (28) at the *Bam*HI site, which was blunt-ended with the Klenow fragment of polymerase I. The recombinant transfer vector pVLVP6C, containing the insert in the correct orientation relative to the polyhedrin promoter, and wild-type AcNPV DNA were cotransfected into Sf9 cells. Recombinant baculoviruses were isolated by limiting dilution, followed by three rounds of plaque purification, as described previously (9). Inoculum was then prepared from the purified recombinant, designated BacVP6C.

A full-length clone corresponding to RNA segment 6 of strain RF (group A) was obtained by synthesizing cDNA

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from double-stranded RNA with two oligonucleotide primers, corresponding to the 5′ and 3′ sequences, respectively, of segment 6 (9). This cDNA was ligated into pVL941, and the selected clone was designated pVLVP6A. Recombinant baculoviruses were obtained by cotransfection with a linear baculovirus genome as described by Kitts et al. (21). Two different inocula of the plaque-purified recombinants were prepared and designated BacVP6A and BacVP6At. The latter, resulting from an abnormal recombination event, expressed a truncated form of VP6A (M_r 36,000 versus 46,000). The DNA of the baculovirus BacVP6At was extracted from infected Sf9 cells, and the region containing the VP6 gene was amplified by polymerase chain reaction with two synthetic oligonucleotides (CGCGCCCGATGGTGGG ACGGTATGAATAATCCGGAATATT and GGGTACGA TGTGGCTCAATGCGGGATCAGA), corresponding to the ends of the polyhedrin gene. The amplification product was purified from agarose as described by Vogelstein and Gillespie (45) prior to sequencing with the Sequenase kit (USB). The recombinant baculovirus BacRF2A, expressing VP2 from the bovine rotavirus (RF strain), has been described previously (22).

Protein analysis. Sf9 cells in 25-cm^2 flasks were infected at high multiplicity (10 PFU/cell). Proteins were labeled at various times postinfection (p.i.) with [^{35}S]methionine (2 μCi/10^5 cells; 800 Ci/mmol) for 2 h in Grace medium, depleted in methionine and containing 1% fetal calf serum. The medium was then removed, and the cells scraped into Grace medium containing the protease inhibitors leupeptin (0.5 μg/ml) and antipain (50 μg/ml). The cells were pelleted at 12,000 × *g* for 1 min and stored at −20°C or used immediately. For protein analysis, 20 μl of clarified medium (corresponding to 2 × 10^4 cells) or 4 × 10^4 cells were dissociated in Laemmli buffer (23) with or without β-mercaptoethanol and analyzed by sodium dodecyl sulfate (SDS)–12% polyacrylamide gel electrophoresis (PAGE) (23). Gels were either stained with Coomassie brilliant blue or fluorographed with Amplify (Amersham), dried, and exposed at −80°C on an X-ray film.

Western immunoblot analysis. Proteins separated by SDS-PAGE were electroblotted onto a Problott membrane (Applied Biosystems). Transfer and immunodetection of proteins were done as described by Labbé et al. (22). Antibodies were either the R50 monoclonal antibody (kindly provided by P. Pothier, Hôpital du Bocage, Dijon, France), which reacts with VP6A, or the BC4 monoclonal antibody, which reacts with VP6C (kindly provided by J. C. Bridger, Institute for Animal Health, Compton, England), both used at a 1:2,000 dilution. A polyclonal serum, kindly provided by J. C. Bridger, which reacts with group C viruses was used at a 1:1,000 dilution.

Purification of the recombinant protein by fast protein liquid chromatography. The pellet of 2 × 10^7 infected cells, harvested at 72 h p.i., was resuspended in 1 ml of 10 mM Tris-HCl (pH 7.5)–0.5% sodium deoxycholate (DOC)–leupeptin (0.5 μg/ml)–antipain (50 μg/ml) and centrifuged for 10 min at 12,000 × *g*. The pellet was resuspended in 1 ml of buffer A (5 M urea, 0.5% Brij 35, 2% betain, 50 mM piperidin, 10 mM dithiothreitol [pH 11.35]) and centrifuged for 10 min at 35,000 rpm (Beckman TL100). The supernatant, diluted to 2 ml with buffer A, was injected into an anion-exchange Mono Q column. Bound proteins were eluted with a salt gradient from 0 to 500 mM NaCl in buffer A. Fractions containing VP6, identified by SDS-PAGE, were pooled and dialyzed extensively against 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.2).

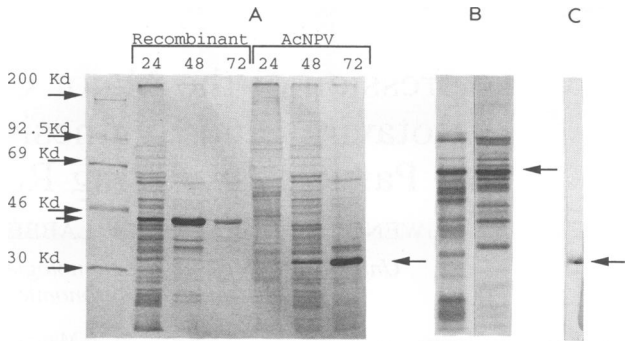

FIG. 1. Comparison of VP6C and polyhedrin expression levels in insect cells and Western blot analysis of the recombinant protein. (A) Proteins from Sf9 cells infected with either a recombinant or a wild-type (AcNPV) baculovirus were labeled at the indicated times (24, 48, or 72 h p.i.) for 2 h with [^{35}S]methionine and resolved by SDS–12% PAGE. The first lane on the left presents standard protein size markers. Polyhedrin and VP6C are indicated by unlabeled arrows on the right and on the left, respectively. (B) Radiolabeled proteins from Sf9 cells infected with BacVP6C (left lane) and proteins present in the culture medium (right lane). VP6C is indicated by an arrow. (C) Proteins from the recombinant-baculovirus-infected cells were separated by SDS-PAGE and transferred to a Problott membrane, and VP6C was detected by a polyclonal antibody. The position of VP6C is indicated by an arrow to the right of the blot.

Effect of pH on purified recombinant protein. The pH of the purified VP6C samples was adjusted to different values (3, 3.5, 4, 4.5, and 5) by addition of sodium acetate buffer (80 mM, final concentration). An aliquot of VP6C from the same preparation was adjusted to pH 2 and centrifuged through Sephadex G25 spun columns equilibrated at pH 3, 3.5, 4, 4.5, or 5 with 100 mM sodium acetate buffer. Samples were analyzed by SDS-PAGE under nondenaturing conditions (i.e., without boiling).

Semipurification of pseudo-single-shelled particles and electron microscopy. Sf9 cells coinfected with BacRF2A and BacVP6C (multiplicity of infection, 10 PFU/cell for each virus) were harvested at 72 h p.i. as described above. The cell pellet was solubilized in 10 mM Tris-HCl (pH 7.4)–antipain (50 μg/ml)–leupeptin (0.5 μg/ml)–1% DOC and clarified by centrifugation for 10 min at 12,000 × *g*. The resulting supernatant was then centrifuged at 50,000 rpm (Beckman TL100) for 30 min. The pellet was dissociated in 10 mM Tris-HCl (pH 7.4) containing protease inhibitors. The semipurified particles present in this pellet were adsorbed onto carbon-coated grids for 2 min. The grids were washed twice with water and stained with 2% uranyl acetate.

RESULTS

Expression of VP6C in *S. frugiperda* cells. The segment 5 coding sequence of group C rotavirus (Cowden strain) was subcloned from a full-length cDNA clone into the transfer vector pVL941. Recombinant baculoviruses were obtained by cotransfection of Sf9 cells with this recombinant vector (pVLVP6C) and wild-type AcNPV DNA as described in Materials and Methods. To analyze the expression of VP6C, infected cells were labeled and harvested at various times postinfection, and the proteins were resolved by SDS-PAGE and compared with those from cells infected with the wild-

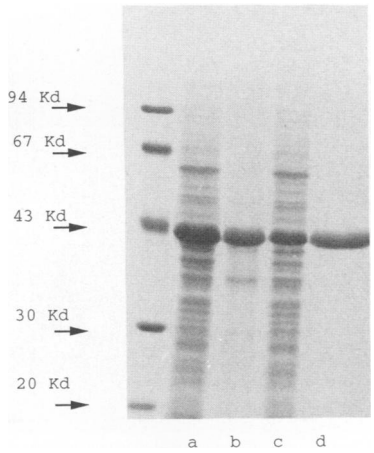

FIG. 2. Purification of the recombinant protein. Proteins from recombinant baculovirus BacVP6C-infected cells harvested at 72 h p.i. were analyzed by PAGE (lane a). In lanes b and c, the proteins obtained after treatment of the infected cells with DOC, as described in Materials and Methods, are shown. The supernatant (lane c) was discarded, and the pellet (lane b) was resuspended in A buffer and used as described in Materials and Methods for fast protein liquid chromatography (FPLC). The fraction corresponding to the VP6C peak is presented in lane d.

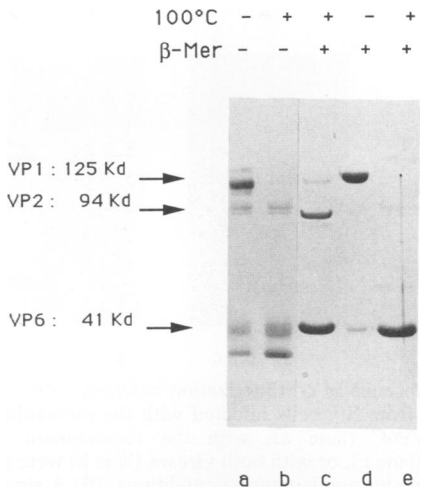

FIG. 3. Oligomeric and tertiary structure of VP6C. The FPLC-dialyzed fraction corresponding to the VP6C peak was solubilized under four different conditions before loading onto an SDS-PAGE gel; under nonreducing conditions at 37°C (lane a) or 100°C (lane b) or under reducing conditions (with β-mercaptoethanol [β-Mer]) at 37°C (lane d) or 100°C (lane e). Purified virus solubilized under reducing conditions was resolved in lane c for comparison, and the molecular masses of the viral proteins are indicated on the left.

type baculovirus (Fig. 1A). In the recombinant-baculovirus-infected cell lysate, polyhedrin disappeared and a new protein with an apparent molecular weight of about 43,000 was detected (Fig. 1A, 48 and 72 h). This protein reacted in a Western blot with an anti-group C rotavirus antiserum (Fig. 1C). VP6C was synthesized as early as 24 h p.i. and up to 72 h p.i. Synthesis was maximum at 48 h p.i. At 72 h p.i., VP6C was found to be approximately twice as abundant in the medium as in the cells (Fig. 1B). As estimated from the Coomassie-stained gel, VP6C is expressed at about the same level as polyhedrin.

Purification of VP6C by anion-exchange chromatography. Treatment of Sf9 infected cells with 0.5% DOC solubilized about 50% of the protein. As shown in Fig. 2, the pellet and the supernatant obtained from infected cells treated with DOC contained equivalent amounts of VP6C (Fig. 2, lanes b and c). However, the ratio of insoluble to soluble protein at this step of the purification process was quite variable. The insoluble fraction contained less cellular protein and thus was used, after solubilization in buffer A, for anion-exchange chromatography. On a Mono Q column, VP6C was eluted in fractions corresponding to 300 mM NaCl and was the only protein detected in these fractions by Coomassie brilliant blue staining. The majority of the protein after this purification step is multimeric (see below).

Oligomerization of VP6C and tertiary structure. The tertiary and quaternary structures of purified recombinant VP6C were investigated by SDS-PAGE under reducing and nonreducing conditions, with and without boiling of the samples. Without boiling before PAGE, most of the monomeric form of VP6C observed in Fig. 3, lane e, disappeared, and a product of high molecular weight appeared (lane d), which corresponds to a trimeric form of VP6C, as will be shown later. The effect of pH on the stability of the trimers of VP6C was investigated. VP6C, purified and dialyzed as described above, was incubated at different pHs, and its structure was examined by SDS-PAGE under nondenaturing conditions. Acidification of the buffer resulted in dissociation of the trimeric form to the monomeric form below pH 4 (Fig. 4). The transformation was reversible, as increasing the pH resulted in the formation of trimers above pH 4. At pH 4, both forms could be detected (Fig. 4).

Analysis of VP6C in the absence of the reducing agent revealed several bands (Fig. 3, lanes a and b) that probably reflected several conformations of the monomer. It suggested the existence of intrachain disulfide bonds, stabilizing the monomeric form of the protein. A similar band pattern was observed in the higher-molecular-weight forms (Fig. 3, lane a). The trimeric form of VP6C was still observed after the addition of a reducing agent, indicating that it did not result from disulfide bonds. Two minor, extra-tight bands of

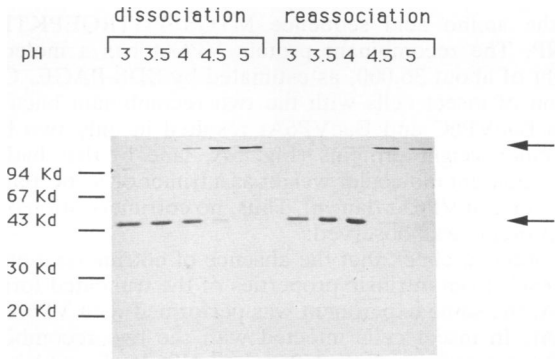

FIG. 4. Dissociation and reassociation of purified (trimeric) VP6C protein. The pH of the VP6C solution was adjusted as described in Materials and Methods; it was first lowered (dissociation) and then increased (reassociation). The samples were analyzed by SDS-PAGE under nondenaturing conditions. The gel was stained with Coomassie brilliant blue. The pH of the sample is indicated at the top of the gel. The positions of VP6C trimers and monomers are indicated by arrows (the upper arrow indicates the trimers). The positions of protein size markers are indicated on the left.

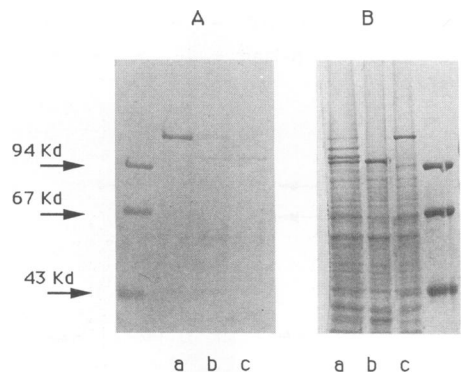

FIG. 5. Absence of cotrimerization between VP6At and VP6C. (A) Proteins from Sf9 cells infected with the recombinant baculovirus BACVP6C (lane a), with the recombinant baculovirus BACVP6At (lane c), or with both viruses (lane b) were analyzed by SDS-PAGE under nondenaturing conditions. (B) A similar analysis was performed with proteins from cells infected with the recombinant baculovirus BACVP6A (lane c), with the recombinant baculovirus BACVP6At (lane b), or with both viruses (lane a). Gels were stained with Coomassie brilliant blue, and the positions of protein size markers are indicated on the left.

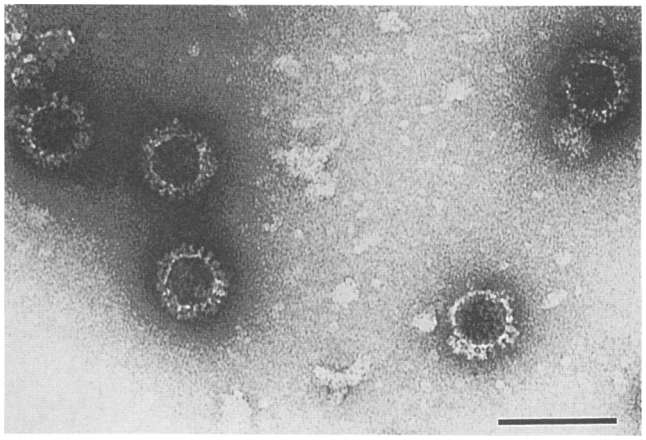

FIG. 6. Negative staining of pseudo-single-shelled particles consisting of VP6C and VP2A. Sf9 cells coinfected with recombinant baculoviruses expressing VP6C and VP2A were solubilized as described in Materials and Methods. Particles were sedimented at 50,000 rpm (Beckman TL100), adsorbed onto carbon-coated grids, stained with 2% uranyl acetate, and examined immediately. Bar, 100 nm.

about 90,000 M_r (lanes a and b) detected in the absence of the reducing agent could represent dimeric forms of VP6C. They could be stabilized by a transitory intermolecular disulfide bond, since they were detected after the samples were boiled under nonreducing conditions, in contrast to the 125,000-M_r polypeptide, which disappeared under the same conditions.

Cotrimerization of different forms of VP6 expressed in the baculovirus system. Since VP6C presented 42% sequence homology with its group A counterpart, the possibility of cotrimerization between VP6C and VP6A proteins was investigated. As VP6 from both groups migrated identically in PAGE gels, we used VP6At, a truncated form of VP6A, to discriminate between the homotrimers and heterotrimers.

The BacVP6At DNA sequence showed that this recombinant baculovirus expressed a fusion protein; the first 300 amino acids are those of VP6A and are followed by 21 amino acids corresponding to the nucleotide sequence of the polyhedrin gene (bases 171 to 230). These extra nucleotides code for the amino acid sequence MTGSFPGTRQEPKTHSL EGNP. The recombinant protein VP6At has a molecular weight of about 36,000, as estimated by SDS-PAGE. Coinfection of insect cells with the two recombinant baculoviruses BacVP6C and BacVP6At resulted in only two high-molecular-weight proteins (Fig. 5A, lane b) that had the same apparent molecular weight as a trimer of VP6C (lane a) or a trimer of VP6At (lane c). Thus, no cotrimerization of the two proteins was observed.

In order to check that the absence of cotrimerization did not result from intrinsic properties of the truncated form of VP6A, the same experiment was performed with VP6A and VP6At. In insect cells infected with the two recombinant baculoviruses expressing VP6A and VP6At, four high-molecular-weight polypeptides were detected (Fig. 5B, lane a). The heavier one corresponded to the oligomeric form of VP6A, present in cells infected with BacVP6A (Fig. 5B, lane c), and the smaller one corresponded to the oligomeric form of VP6At, present in cells infected with BacVP6At (Fig. 5B, lane b). The other two products migrating between these two bands were cotrimers of VP6A and VP6At. In both cases, the authenticity of the VP6 forms was confirmed by Western blot analysis (data not shown). The presence of four oligomeric forms of VP6 in the coinfected cells clearly showed that the oligomeric form of VP6 is a trimer.

Assembly of pseudo-single-shelled particles by coexpression of VP2A and VP6C in Sf9 cells. Negative staining of a lysate of Sf9 cells infected with BacVP6C revealed tubules and honeycomb structures similar to those described for the group A counterpart. Coinfection of Sf9 cells with BacRF2A and BacVP6C resulted in the formation of particles similar to single-shelled virions (Fig. 6). However, a high percentage of the particles observed by electron microscopy had a heterogeneous morphology. Most of them consisted of a corelike particle surrounded by an incomplete layer of VP6C capsomers. Particles consisting only of VP2, corresponding to previously described pseudocores (22) completely devoid of the VP6 outer layer, were not observed in preparations resulting from coinfection. The diameter of particles having a uniform shell of VP6 is about 60 nm. Structures evoking trimers were also observed. Coinfection of Sf9 cells with baculoviruses expressing VP2 and the truncated form of VP6A resulted in corelike particles, but interaction between these two proteins could not be evidenced by electron microscopy.

DISCUSSION

Several studies have recently given insight into the structure of non-group A rotavirus (2, 7, 14, 20). Despite progress in the adaptation of these viruses to growth in tissue culture (39, 46), the availability of viral antigen for the preparation of diagnostic reagents is still limited. In group A rotaviruses, VP6 is a highly immunogenic protein, and since VP6 is also the major group antigen (32), antibodies directed against this protein are very useful reagents for diagnosis. Limited information is available on the antigen characteristics of non-group A viruses; however, it can be expected that the corresponding protein also bears an important group epitope. Thus, a recombinant VP6 could help in developing diagnostic kits. The group C (Cowden strain) protein VP6 has been expressed to high levels in the baculovirus expres-

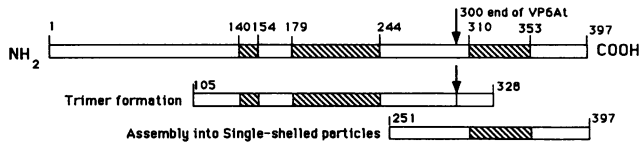

FIG. 7. Domains of VP6 involved in trimerization and assembly into single-shelled particles. Regions of high divergency between VP6A and VP6C are indicated by a hatched box. The end of homology between VP6A and VP6At is indicated by an arrow. Numbering of amino acids refers to the VP6A sequence. This figure is derived from results obtained by Clapp and Patton (8).

sion system. The recombinant protein is similar in size to the native viral protein and is able to react with monoclonal and polyclonal antibodies raised against the corresponding rotavirus. Taking advantage of the partial insolubility of the recombinant protein, which contrasts with the solubility observed with the group A VP6 also expressed in the baculovirus system (12), we could easily obtain large quantities of purified antigen.

Well-characterized molecular weight markers of sufficiently large size are not available for SDS-PAGE, and some discordant data have been published on the trimeric or tetrameric self-assembly of VP6 of groups A, B, and C (16, 20). The use of a truncated mutant of the group A protein allowed us to conclude unequivocally that the larger form of VP6, which is resistant to SDS, is trimeric. The comigration of VP6 multimers of both groups suggests that the group C protein is also trimeric. We also observed a minor dimeric form of the protein, stabilized by disulfide bonds. This form had already been described for purified particles of group A virus (38). It could represent either a step in the formation of the trimers or an abnormal oligomerization which does not allow complete oligomerization to occur. Comparison of the VP6 amino acid sequences of the two virus strains used here confirms the possible existence of a disulfide bond in the monomeric form of VP6, since both strains contain three cysteines, one of which is conserved and the other two of which are located in the same region. It can be noted that trimers of the adenovirus type 2 fiber also resist SDS at a low temperature and are not stabilized by covalent bonds. Adenovirus type 2 fiber also assembles in trimers and presents a dimeric form, which could be a limiting step for the assembly of trimers (31). The sequence of adenovirus type 2 fiber, like the sequences of VP6, is predicted to be rich in β structures. It can be hypothesized that these β structures are implicated in the formation of the trimers.

The high sensitivity of VP6 quaternary structures to pH had already been demonstrated by electron microscopy. Lowering the pH leads to the disaggregation of tubules, which convert into round particles or hexamers (35). We probably observed the same phenomenon, which is reversible in our experiments, for VP6C trimers that become monomeric. Electron microscopy of purified VP6C preparations allowed us to observe larger forms than the trimeric ones and, in particular honeycomb sheets, but no round particles were seen. However, by SDS-PAGE, we were not able to observe larger forms than the trimeric ones, since they are probably not resistant to SDS. The formation of trimers does not seem to be dependent on significant amounts of cellular proteins, since purified VP6C could trimerize in vitro after a simple change of pH. This result makes a role for cellular proteins, e.g., chaperones, in the assembly of trimers very unlikely.

In vitro translation of truncated proteins has permitted delineation of a region needed for trimerization (8) of group A VP6. The amino acid sequences of the VP6 genes used in this study show 42% homology. The absence of cotrimers between VP6A and VP6C suggests that the interactive sites involved in VP6 trimerization are probably not conserved, although each protein is able to trimerize itself. Comparison of the sequences of VP6A (RF strain) and VP6C (Cowden strain) showed that, while the amino-terminal end (1 to 140) exhibits conservation, two domains in the central region (140 to 154 and 179 to 244) and a domain near the carboxy-terminal end (310 to 353) are highly divergent. Thus, regions 140 to 154 and 179 to 244, which are included in the domain previously found to be essential for trimerization (amino acids 105 to 328) (8) (Fig. 7), could contain group-specific structures that prevent heterotrimerization. Sequencing of the recombinant BacVP6At DNA showed that VP6At differs from VP6A from amino acid 300. This observation may indicate that the domain essential for trimerization found by Clapp and Patton ends at amino acid 300 instead of amino acid 328 (Fig. 7). Trimers of VP6 are stabilized by noncovalent interactions that resist SDS at 37°C. The VP6 sequence is very hydrophobic, particularly so at amino acids 20 to 90 and 150 to 370. It can be hypothesized that hydrophobic interactions are responsible for the stability of trimers. Disulfide bonds did not play a direct role in the stability of trimers that resist high concentrations of β-mercaptoethanol; however, the existence of an intrachain disulfide bond is strongly suggested by the migration changes of reduced and nonreduced trimers and monomers. The fact that trimer bonds between VP6 proteins are sensitive to pH 3.5 whereas VP6 remains associated with cores at pH 2.5 (data not shown) confirms previous indications that trimerization of VP6 is not a prerequisite for the assembly of single-shelled particles (8).

This article presents the first report on the assembly of recombinant rotavirus pseudo-single-shelled particles containing the VP2 and VP6 proteins of different serogroups. These results indicate that the absence of reassortants between these viruses is probably not due to the impossibility of assembly of the inner capsid proteins. In insect cells, the group C VP6 was able to interact with the group A VP2 to form chimeric single-shelled particles having the same size and morphology as group A single-shelled particles. Similar chimeric particles have been obtained by coexpression in insect cells of bluetongue virus and epizootic hemorrhagic disease virus proteins (24). However, these two orbiviruses are serologically related, which is not the case for rotaviruses of different serogroups. Assembly of VP6 group C with VP2 group A demonstrates conservation of group A and group C VP6 protein structures, at least regarding interactions with the VP2 protein, which is the major component of the cores. In terms of primary structure, one or several of the conserved regions might be involved in these interactions. It is worthwhile noting that two regions (positions 251 to 310 and 353 to 397) are located in the region of VP6 group A (amino acids 251 to 397) that is essential for binding to single-shelled particles (8). Further studies are still needed to identify the sites of interaction involved in particle assembly within these conserved regions. The ability to purify large amounts of both recombinant VP6 and pseudo-cores will probably help in elucidating the nature of the interactions.

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