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Synthesis and Immunogenicity of the Rotavirus Major Capsid Antigen Using a Baculovirus Expression System

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Rotaviruses are the major pathogens that cause life-threatening diarrhea in young children and animals. We inserted a simian rotavirus SA11 gene 6 cDNA into the genome of the baculovirus *Autographa californica* nuclear polyhedrosis virus adjacent to the strong polyhedrin promoter. The major capsid antigen (VP6) was expressed in high yields (20 to 150 μg/10^6 cells) when *Spodoptera frugiperda* cells were infected with baculovirus recombinants containing SA11 gene 6 inserts. Reactivity with monospecific polyclonal and monoclonal antibodies suggested that VP6, expressed intracellularly or found in the media, maintained native antigenic determinants. VP6 purified from the media from infected cells also possessed a native oligomeric structure, was immunogenic in guinea pigs, and was able to spontaneously assemble into morphologic subunits. Antisera from immunized guinea pigs failed to neutralize virus in plaque reduction assays, but detected homologous and heterologous rotavirus strains when tested by immunofluorescence, immunoprecipitation, and enzyme-linked immunosorbent assays.

The importance of rotaviruses as etiologic agents of infantile diarrhea in humans and animals is now clearly established (10, 14). The recognition that the rotaviruses are the major cause of life-threatening diarrheal disease and of significant morbidity in the young has focused efforts on disease prevention and control against these viruses.

Studies on rotavirus gene structure and function are expected to help these efforts. For example, the cloning and subsequent expression of individual protein products are helping elucidate the function of each protein in virus structure, replication, and assembly. Analysis of the antigenic, functional, and molecular properties of expressed gene products, alone or in conjunction with other viral proteins, will also allow intrinsic properties of each gene product to be determined. In addition, humoral and cell-mediated immune response(s) to specific viral proteins will be more easily and directly dissected with the availability of high levels of individual proteins. Finally, the availability of large amounts of immunogenic structural proteins will facilitate vaccine testing and the production of inexpensive diagnostic tests.

The genome of rotaviruses consists of 11 segments of double-stranded RNA. The genome RNA is enclosed within a double-layered protein capsid that is made up of the structural proteins VP1 to VP9 (8). Each genome segment encodes at least one protein (8, 17), and knowledge of the properties of the structural proteins is increasing. The outer capsid proteins (VP3 and the glycoprotein VP7) function as the viral hemagglutinin (H antigen, VP3) and neutralization (N antigen, VP7) antigens. VP3 also induces neutralizing antibodies. The major capsid protein that comprises >80% of the protein mass of the particle is located on the inner capsid and contains the subgroup antigen (S antigen, VP6) (8). The presence of VP6 on virus particles has been associated with viral RNA polymerase activity (4), and VP6 may bind to RNA genome segments or transcripts or interact with other viral proteins during replication and assembly (8). VP6 has recently been reported to possess an oligomeric (possibly trimeric) conformation (12), and it is also the major protein detected in diagnostic enzyme-linked immunosorbent assays (ELISA) (3). Neutralizing and protective antibodies are produced to the outer capsid H and N antigens (21), but information on whether other structural proteins (VP1, 2, 6, and 9) play a role in inducing protection from infection is less clear.

We report the successful production of the major capsid antigen (VP6) of the simian rotavirus SA11 in insect cells, using the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) as an expression vector. VP6 was produced efficiently, and the majority of the protein was found in the media from infected cells. Immunologic and biochemical analysis indicated that the protein possessed both native antigenic determinants and an oligomeric conformation. The availability of large amounts of VP6 will help determine intrinsic biochemical and functional properties of this protein in the rotavirus replication process, including viral morphogenesis.

MATERIALS AND METHODS

Cells and virus. The simian rotavirus SA11 was grown in cultures of MA104 cells as previously described (9). The baculovirus *A. californica* (AcNPV) and the recombinant virus pAc461/SA11-6 were used to infect *Spodoptera frugiperda* (IPLB-SF21-AE) cells at a multiplicity of infection of 10 PFU per cell. *S. frugiperda* cells were grown and maintained on Hink's medium containing 10% fetal bovine serum (FBS) (26).

Construction and selection of baculovirus recombinants containing SA11 gene 6 cDNA inserts. SA11 gene 6 was originally cloned in the *Pst*I site of pBR322 (10). The resulting clone, pSA11-6, was digested with *Aha*III and *Hpa*II and subcloned into the *Sma*I site of the baculovirus transfer vector pAc461. Plasmid pAc461 was derived from

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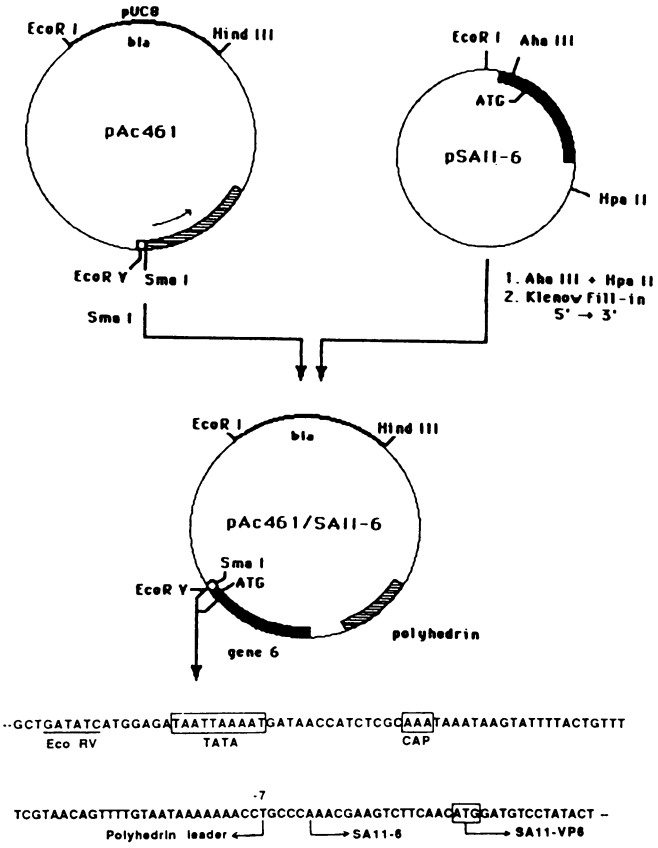


FIG. 1. Construction of the baculovirus expression vector for rotavirus SA11 gene 6 synthesis. The full-length SA11 gene 6 cDNA, pSA11-6, was digested with *Aha*III and *Hpa*II and filled in with the Klenow fragment. Purified insert DNA was cloned into the *Sma*I site of pAc461 by blunt-end ligation. The resultant vector, pAc461/SA11-6, contains the SA11 gene 6-coding region fused to the polyhedrin leader sequence. The TATA region, the CAP site for transcription initiation, and the ATG initiation codon of the chimeric gene are boxed. Symbols: □, polyhedrin promoter region; ▨, polyhedrin-coding sequences; ▬, SA11 gene 6 sequences; —, pBR sequences; ——, AcNPV sequences. The baculovirus pAc461 vector was derived from pAc311 by cleavage with *Bam*HI and *Kpn*I, digestion with Bal 31 nuclease, and ligation of a *Sma*I linker. This vector has a deletion between −7 and +670 within the polyhedrin gene.

pAc311 (26) by cleavage with *Bam*HI and *Kpn*I, digestion with Bal 31 nuclease, treatment with DNA polymerase I (Klenow fragment) to produce blunt ends, ligation of a *Sma*I (GCCCGGGC) linker, cleavage with *Sma*I, and religation. Plasmid pAc461 has a deletion between positions −7 and +670 in the baculovirus polyhedrin gene. After transfection into *Escherichia coli*, plasmids in recombinant ampicillin-resistant colonies were screened by restriction enzyme analysis for inserts in the correct transcriptional orientation. One of these, designated pAc461/SA11-6, is missing the first seven nucleotides of the 5′ end of SA11 gene 6 and has about 70 extra base pairs of tails that were added at the 3′ end during the original cloning into pBR322 (Fig. 1). Transfer of SA11 gene 6 cDNA from this vector to the AcNPV genome DNA was achieved by cotransfection of *S. frugiperda* cells with wild-type AcNPV DNA, using the calcium phosphate precipitation procedure as previously described (26). Briefly, the AcNPV DNA (1 μg) was mixed with 2 μg of pAc461/SA11-6 DNA, brought to 950 μl with HEBS/CT (0.137 M NaCl, 6 mM D-glucose, 5 mM KCl, 0.7 mM $Na_2HPO_4 \cdot 7H_2O$, 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 15 μg of sonicated calf thymus DNA per ml, pH 7.1) and vortexed. While the mixture was being slowly vortexed, 50 μl of 2.5 M $CaCl_2$ was added, and a precipitate was allowed to form at room temperature for 30 min. The pecipitated DNA was added to 2 ml of Hink's medium supplemented with 10% FBS in a 25-cm^2 flask seeded with 2.5×10^6 *S. frugiperda* cells. Following incubation at 27°C for 4 h, the medium was removed, the monolayer was washed with fresh Hink's medium containing 10% FBS, and, after addition of 5 ml of Hink's medium supplemented with 10% FBS, the flask was incubated at 27°C. The cells were observed with an inverted microscope for signs of infection, and, at an advanced stage of infection (day 5), extracellular virus was harvested and plaqued on monolayers of *S. frugiperda* cells. Recombinants, in which the polyhedrin gene had been replaced by the polyhedrin-SA11-6 transfer vector DNA by homologous recombination, were selected by identifying occlusion-negative plaques with an inverted phase microscope (26). Virus in occlusion-negative plaques was plaque purified three times and used to propagate virus stocks. Three recombinant virus stocks, designated pAc461/SA11-6.1, pAc461/SA11-6.2, and pAc461/SA11-6.3, were used for further analyses.

Radiolabeling of proteins synthesized in infected *S. frugiperda* cells. *S. frugiperda* cells infected with recombinant/SA11-6 or with wild-type AcNPV were radiolabeled at different times postinfection with 15 μCi of [^{35}S]methionine per ml in methionine-free Hink's medium supplemented with 10% FBS. The cells and media were harvested at 42 h postinfection, and the cells were pelleted at $1,400 \times g$ at 4°C for 5 min. The cell-free supernatant was removed and saved for testing, and the cell pellet was suspended in RIPA buffer (0.15 M NaCl, 0.01 M Tris hydrochloride [pH 7.2], 1% aprotinin, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate). Proteins were immunoprecipitated from these samples as previously described (5). Antisera used for immunoprecipitation were a polyclonal guinea pig anti-SA11 serum (gp αSA11) that reacts with SA11 structural proteins VP2, 3, 6, and 7 (7) or ascites fluids from mice injected with hybridoma cells (produced by Harry Greenberg and kindly provided by Tom Flewett, World Health Organization Collaborating Centre for Rotaviruses, Birmingham, U.K.) that secrete monoclonal antibodies to VP6 subgroup I (255/60, αSGI) or subgroup II (631/9, αSGII) or to a common determinant on VP6 (631/7, αcommon) (13). The specificity of these monoclonal antibodies was demonstrated by ELISA and by immunoprecipitation assays with human and animal rotavirus strains (13; M. K. Estes, S. E. Crawford, and M. E. Penaranda, unpublished data). In some cases the immunoreactivity of the expressed protein was also analyzed by immunoblots (J. W. Burns and M. K. Estes, manuscript in preparation).

Partial purification of the expressed rotavirus VP6. Recombinant-infected *S. frugiperda* cells were labeled 28 h postinfection with [^{35}S]methionine (30 μCi/ml, 1,200 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) in Hink's medium lacking FBS. The cells and media were harvested separately between 90 and 106 h postinfection by scraping cells into the media and pelleting the cells by centrifugation at 1,000 rpm for 20 min at 4°C in a Damon IEC centrifuge. In some cases, VP6 was purified from the media by centrifuging clarified ($100,000 \times g$ for 30 min) media containing VP6 on a 5 to 20%

continuous sucrose gradient for 23 h at 100,000 × *g*. Fractions containing the expressed VP6 that lacked contaminating bovine serum albumin were pooled, dialyzed against 10 mM Tris hydrochloride (pH 7.5), and used either directly or after lyophilization.

In some experiments, wild-type AcNPV and recombinant expressed proteins were prepared by using the same infection conditions and harvesting procedure, but were not purified further. In this case, medium from infected cells was collected, dialyzed against 10 mM Tris hydrochloride (pH 7.5), and used directly or lyophilized. Concentrations of VP6 were approximated by comparing the amounts of VP6 to quantitative marker proteins after electrophoresis on 12% polyacrylamide gels and staining with silver nitrate or Coomassie blue or by a quantitative ELISA.

Affinity purification of expressed VP6. Protein A-Sepharose CL-4B affinity columns (Pharmacia, Inc., Piscataway, N.J.) were prepared by cross-linking subgroup I monoclonal supernatant to the gel, using dimethylpimelimidate dihydrochloride (25). Unlinked antibody was removed by washing with 0.1 M borate buffer (pH 8.3). Supernatants from *S. frugiperda* cells infected with pAc461/SA11-6.1 were mixed with the immunomatrix, shaken at room temperature, and centrifuged at 1,500 rpm for 1 min in a Damon IEC centrifuge. The immunomatrix gel was then suspended in borate buffer and poured into a disposable Econocolumn (Bio-Rad Laboratories, Richmond, Calif.). The column was washed sequentially with 50 ml each of buffer A (0.5 M NaCl, 0.05 M Tris hydrochloride [pH 8.2], 0.5% Nonidet P-40), buffer B (0.15 M NaCl, 0.05 M Tris hydrochloride [pH 8.2], 0.5% Nonidet P-40, 0.1% sodium dodecyl sulfate), and buffer C (0.15 M NaCl, 0.5% sodium deoxycholate), with borate buffer washes between each. After a final wash with borate buffer, VP6 was eluted from the gel with 0.1 M glycine-HCl (pH 2.5) into tubes containing 2 M Tris base to neutralize the acid. The eluted VP6 was analyzed by electrophoresis on 12% polyacrylamide gels and was used as a standard in ELISA to quantitate the kinetics and levels of expression of VP6.

Production of antiserum to expressed VP6. Guinea pigs (Elm Hill Breeding Farms, Chelmsford, Mass.) shown to lack rotavirus antibodies were used for the production of antiserum to expressed VP6 protein. Each guinea pig was inoculated intramuscularly twice at 3-week intervals as previously described (9). The antigens used were partially purified expressed VP6 or proteins concentrated from the supernatant from *S. frugiperda* cells infected with either AcNPV or pAc461/SA11-6. The guinea pigs were bled 1 week after the second injection of antigen, and the serum samples were tested for the presence of antibodies by immunofluorescence, immunoprecipitation, ELISA, and immune electron microscopy assays as previously described (5, 9, 20).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein products were analyzed by polyacrylamide slab gel electrophoresis, using a modification of the method of Laemmli with 12% separating and 4% stacking gels as previously described (5). Before electrophoresis, unless otherwise stated, samples were dissociated by boiling for 3 min in sample buffer containing 1% sodium dodecyl sulfate, 10% 2-mercaptoethanol, 0.5 M urea, 0.05 M Tris hydrochloride (pH 6.8), 10% glycerol, and 0.0025% phenol red. Radiolabeled proteins were monitored on gels following fluorography as previously described (17).

Electron microscopy. Supernatant from *S. frugiperda* cells infected with pAc461/SA11-6.1 which had been dialyzed into 10 mM Tris hydrochloride, pH 7.5, was diluted with an equal volume of 0.3 M NaCl–20 mM EDTA, pH 6.0, and kept at 4°C for several weeks. Samples for electron microscopy were adsorbed onto carbon-collodion-coated grids and negatively stained with 2% aqueous uranyl acetate. Reagents for immunogold labeling were prepared by direct coupling of αSGI and monoclonal antibody against the nonstructural glycoprotein (αNS29) to 7-nm colloidal gold particles as described previously (6). After adsorption of the sample to the grid, labeling was accomplished by floating the grids on 20-μl drops of the appropriate antibody-gold complex for 30 min at room temperature. Grids were then washed in distilled water and stained with uranyl acetate.

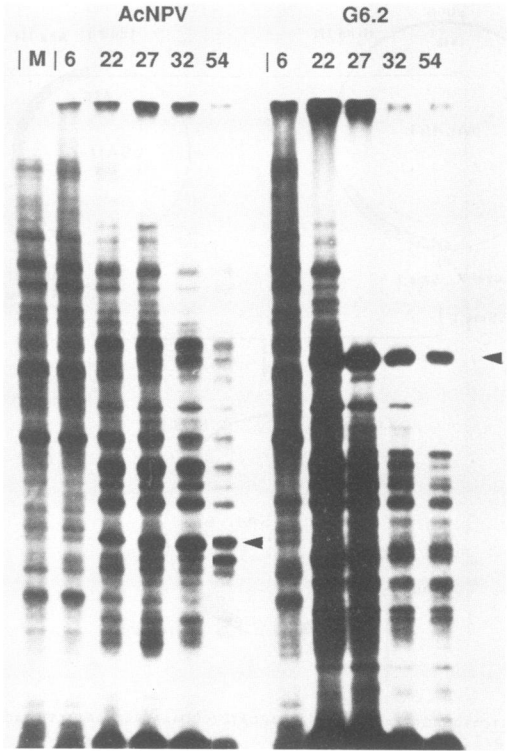


FIG. 2. Kinetics of VP6 synthesis in infected *S. frugiperda* cells. The proteins synthesized in mock (M)-, wild-type baculovirus (AcNPV)-, or baculovirus recombinant (SA11 gene [G] 6.2)-infected *S. frugiperda* cells were labeled with [^{35}S]methionine (30 μCi/ml) at the indicated times (in hours). The proteins in cells harvested 2 h later were then analyzed in 12% sodium dodecyl sulfate-polyacrylamide gels. Polyhedrin and SA11 VP6 are indicated by arrowheads in the left- and right-hand panels, respectively.

RESULTS

Expression of SA11 VP6 in *S. frugiperda* cells. Three baculovirus recombinants (6.1 to 6.3) containing an SA11 gene 6 cDNA insert were initially identified and tested for their ability to produce SA11 VP6 after infection of *S. frugiperda* cells. The [^{35}S]methionine-containing polypeptides in cells infected with AcNPV or SA11 gene 6 recombinant are shown in Fig. 2. A new band of the expected molecular weight (41,000) was seen in recombinant-infected cells, while the polyhedrin protein was only seen in AcNPV-infected cells. Radiolabeled VP6 was initially detected at 22 h postinfection, and its synthesis was still detectable at 105 h postinfection (see below).

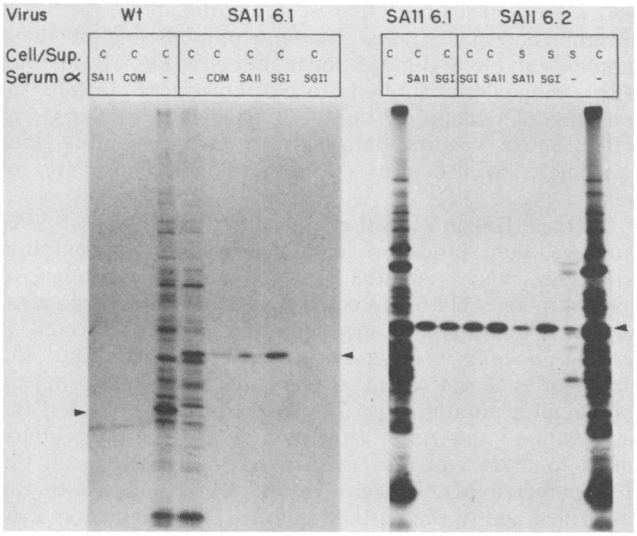


FIG. 3. Immunoreactivity of SA11 VP6 expressed in *S. frugiperda* cells. [^{35}S]methionine-labeled proteins synthesized in wild-type- or SA11 gene 6 recombinant (SA11 6.1 or SA11 6.2)-infected *S. frugiperda* cells. AcNPV polyhedron (▶) and SA11 VP6 (◀) are highlighted. The immunoreactivity of these proteins with antiserum to SA11 particles (αSA11) or with monoclonal antibodies to epitopes present (αSGI, COM) or absent (αSGII) on SA11 VP6 or with no serum (−) is shown. The left-hand panel shows reactivity of cell-associated VP6, while the panel on the right shows reactivity with VP6 from cells (C) or from the medium supernatant (S) from cells infected with two different gene 6 recombinants. The weaker reactivity shown between VP6 from the supernatant and SA11 serum (compared with the SGI reactivity) was not reproducible; other experiments showed similar strong positive reactivity with all three antisera and VP6 in the supernatant.

Immunoreactivity and localization of expressed VP6. The 41,000-molecular-weight protein (41K band) was identified as authentic VP6 by positive immunoreactivity with polyclonal antisera prepared to SA11 virus and with monoclonal antibodies to two epitopes (common and subgroup I) on the SA11 VP6 (Fig. 3). Specificity of these reactions was shown by a lack of reactivity of the subgroup II antibody with the SA11 41K protein and by finding that no proteins in AcNPV-infected cells reacted with any of these sera. Similar results were obtained with all three recombinants.

We also examined the cellular localization and levels of expression of VP6. VP6 was observed in the cytoplasm of recombinant-infected cells by immunofluorescence (data not shown), and it could be immunoprecipitated from both clarified supernatants and cell-associated proteins (Fig. 3). The amount of protein being synthesized was quantitated by analysis of dilutions of immunoprecipitated VP6 (data not shown) and by ELISA (Fig. 4) standardized with VP6 purified to radiochemical and biochemical homogeneity from an immunomatrix column. Results from such analyses estimated that cell-associated VP6 was produced in infected cells in amounts of 20 to 150 μg from a density of 10^6 cells per ml. The ELISA results also showed that immunoreactive VP6 could be detected as early as 8 h postinfection and that the amount of VP6 found in the medium increased with time. Yields of VP6 in clarified media were sufficient to allow purification for further biochemical and immunologic studies. Yields of VP6 were greatest when cells were grown in complete media containing 10% FBS.

Conformational properties of expressed VP6. Gorziglia et al. (12) have presented evidence that VP6 removed from purified bovine rotaviruses or analyzed in infected cells behaves as an oligomeric (possibly trimeric) protein. This experiment offered an independent way to examine whether expressed SA11 VP6 possessed conformational properties similar to authentic VP6. Figure 5 shows that the profile of [^{35}S]methionine-labeled proteins in purified single-shelled virus after electrophoresis in SDS-polyacrylamide gels was different depending on whether or not the virus was heated before the gels were loaded. In the presence of heating, characteristic bands representing VP1 (125K), VP2 (94K), and VP6 (41K) were seen. In the absence of heating, the VP6 band disappeared and a new band migrating above VP1 was observed. These control data have extended the observations of Gorziglia and co-workers to SA11 (another rotavirus strain). Similar analyses with baculovirus-expressed VP6 (partially purified on sucrose gradients) and with immunoaffinity-purified VP6 showed that these proteins also formed oligomers (Fig. 5). In the absence of reducing agents (2-mercaptoethanol), an additional band that also migrated as an oligomer was observed. Monomeric and oligomeric forms of expressed VP6 were also seen by using immunoblots. These analyses confirmed that the expressed VP6 possessed a native conformation and showed that oligomer formation must be an intrinsic property of VP6. The expressed VP6 also assembled spontaneously into tubules after several weeks at 4°C (Fig. 6). These tubules showed a hexagonal array of subunits that resembled those which have been observed in stool samples from children and animals infected with rotavirus. The hexagonal subunit pattern on the tubules

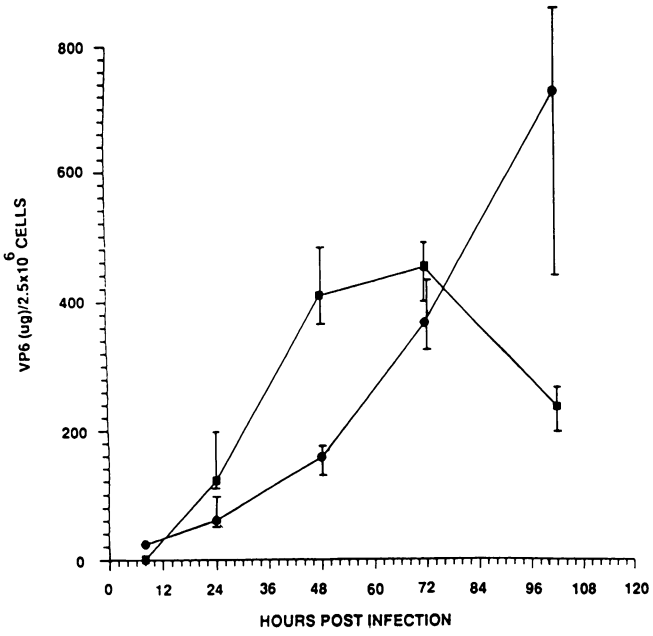


FIG. 4. Detection of VP6 expression by ELISA. ELISA results showing amounts of VP6 detected by ELISA in media (●) or in SA11-infected *S. frugiperda* cells (■) at the indicated times postinfection. The amounts of VP6 at each time-point were quantitated by direct comparison of the optical density (OD) readings of SA11 6.1-infected cell samples with the OD values on a standard curve of a known amount of affinity-purified VP6. Background OD values (0.001 to 0.016) from mock and wild-type AcNPV-infected cells were subtracted. The range of amounts of VP6 detected in different infections is shown.

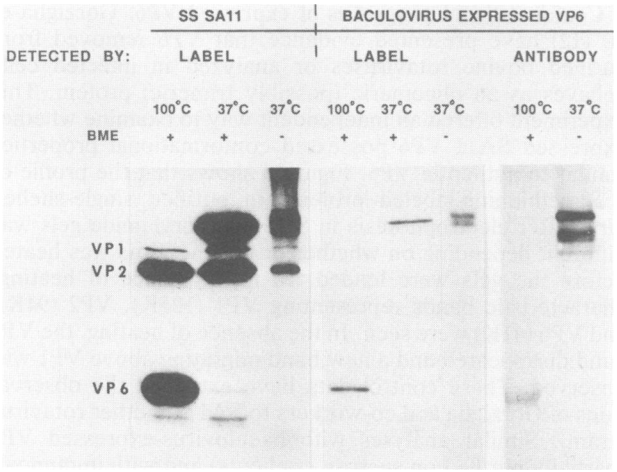
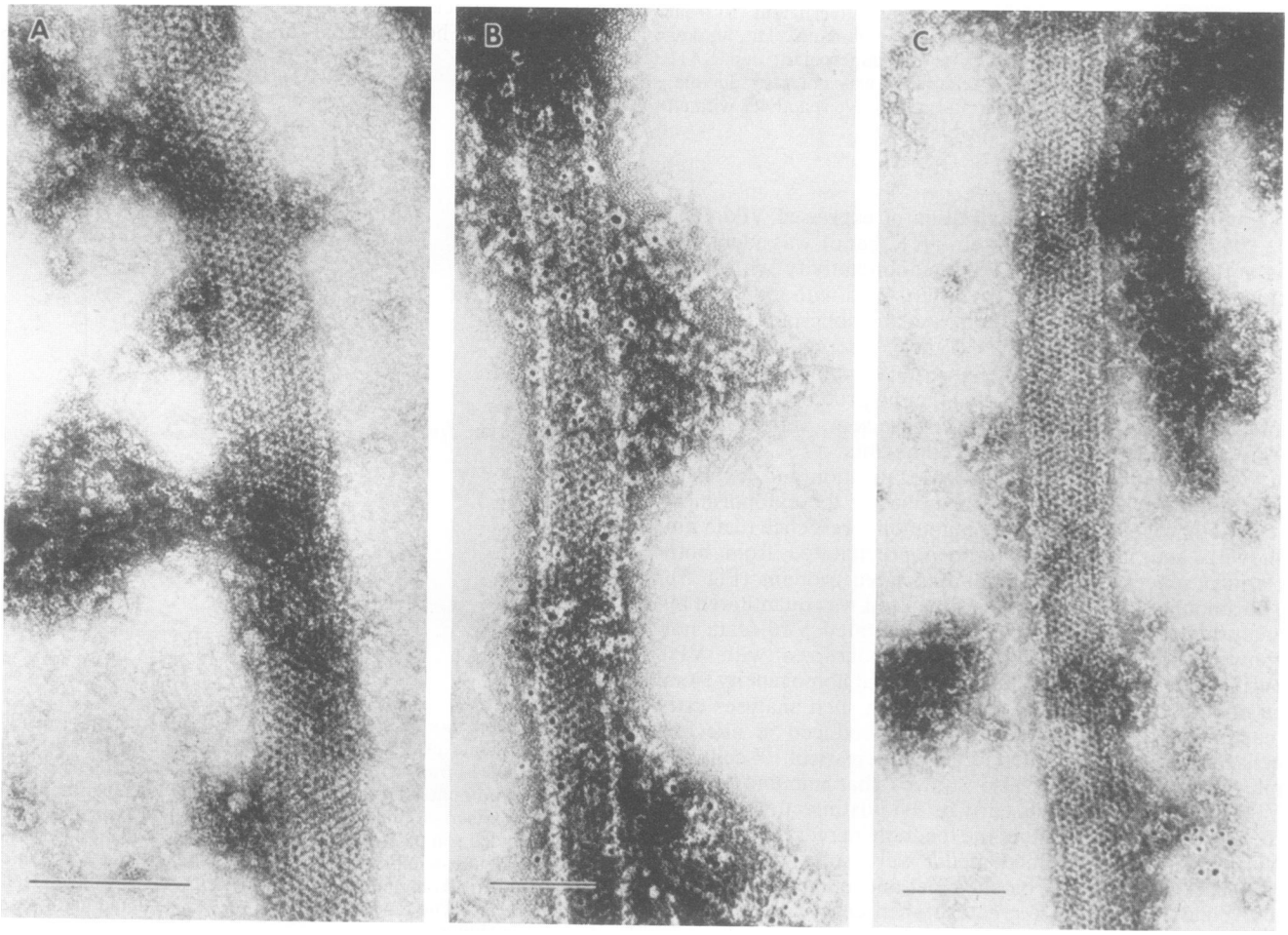


FIG. 5. Comparison of conformation of expressed and authentic VP6. The polypeptides in single-shelled (SS) SA11 particles or in baculovirus-expressed VP6 were separated on 12% polyacrylamide gels after heating at either 100°C for 2 min or 37°C for 30 min in sample buffer in the presence (+) or absence (−) of β-mercaptoethanol (BME). The gels were processed by fluorography and autoradiography to detect radiolabeled proteins, or the separated proteins were electrophoretically transferred to nitrocellulose and then detected by using the SGI monoclonal antibody.

also closely resembled that seen on the inner capsid of several rotavirus strains. Immunogold labeling of these tubules with the SGI monoclonal antibody (Fig. 6B) and not with a monoclonal antibody to the nonstructural glycoprotein (Fig. 6C) confirmed that the tubules were composed of VP6. These results demonstrate that assembly into capsomerelike structures is another intrinsic property of VP6.

Characterization of antisera produced to expressed VP6. Antisera were produced in guinea pigs to concentrated wild-type AcNPV-infected *S. frugiperda* cell proteins, to concentrated SA11-6.1-infected *S. frugiperda* cell proteins, and to VP6 partially purified from the media of infected *S. frugiperda* cells by sucrose gradient centrifugation. The ability of each antiserum to recognize VP6 was tested by immunoprecipitation (Fig. 7). All preimmune sera and the antiserum to the AcNPV-infected *S. frugiperda* proteins failed to react with any cellular or viral proteins in the SA11-infected MA104 cell lysates, while antisera to the unpurified and to the partially purified VP6 reacted specifically with VP6. These antisera could also be used for virus detection. The anti-VP6 sera detected rotavirus strains representing each known human serotype and subgroup in both immunofluorescence tests and ELISA (data not shown). The anti-VP6 sera did not neutralize SA11 infectivity when tested in plaque reduction neutralization assays with 50 PFU of virus.



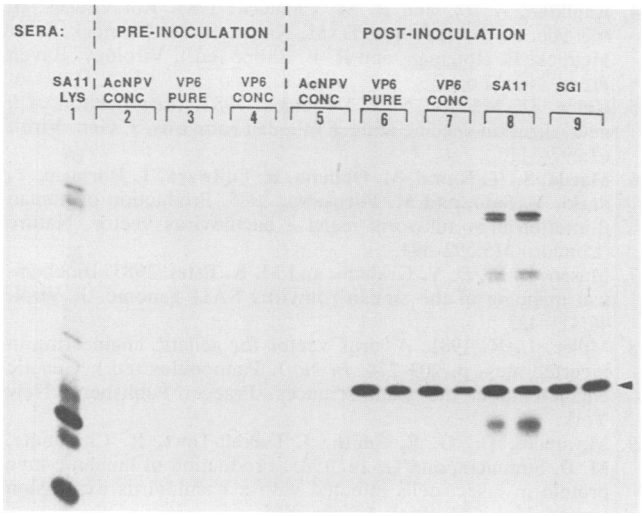


FIG. 7. Characterization of guinea pig antiserum produced to expressed VP6. [^{35}S]methionine-labeled polypeptides in SA11-infected MA104 cells (lane 1) were immunoprecipitated with guinea pig antiserum made to concentrated proteins from wild-type baculovirus (AcNPV)-infected *S. frugiperda* cells (lane 5); VP6 purified from recombinant-infected *S. frugiperda* cells (lane 6); concentrated (but unpurified) proteins from SA11-6.1-infected *S. frugiperda* cells (lane 7); or purified double-shelled SA11 (lane 8). Lane 9 shows VP6 immunoprecipitated with monoclonal antibody to the SGI epitope on VP6. Preimmune serum from all animals showed no reactivity with any proteins from these lysates (lanes 2 to 4). Viral proteins VP2, VP3, VP5, VP6, and VP7 that react with antiserum to double-shelled virus are seen in lane 8; VP6 is highlighted with an arrow. Lanes 2 to 9 show analyses performed with undiluted or a 1:10 dilution of the indicated serum.

DISCUSSION

The production of viral proteins in high yields from expression vector systems offers new ways to study viral protein function and to develop diagnostic tests and vaccines. We have evaluated the antigenic and molecular properties of the simian rotavirus SA11 major capsid protein VP6, produced by using a baculovirus expression system. Several properties of the expressed VP6 are noteworthy. First, although VP6 is not a glycoprotein and is not found in the media from SA11-infected cells, it was present in the media from the baculovirus recombinant-infected cells. The current study did not determine whether VP6 was actively secreted or was released only as a result of cell lysis, but its presence in the media greatly facilitated the purification of VP6. Other nonglycosylated proteins, β-galactosidase and chloramphenicol transferase, have also been found in the media after production with the same baculovirus expression vector (G. E. Smith and M. D. Summers, unpublished observation).

Second, VP6 was expressed in its native conformation. Reactivity with available monoclonal antibodies suggested that native immunoreactive determinants were conserved. Further evidence of a native conformation was provided by the demonstration that the expressed (and purified) protein was an oligomer, the reported structure of VP6 in virus-infected cells or in virus particles (12). This observation strongly suggests that oligomer formation is an intrinsic property of this protein. However, our data differ from those of Gorziglia et al. (12), as we observed that the formation of multiple oligomeric forms is associated with disulfide bonds. Although seen with radiolabeled VP6, this property of VP6 was observed more clearly by using a sensitive protein-blotting procedure to detect the oligomers.

Electron microscopic analysis revealed that further assembly of the trimeric VP6 molecules into morphologic subunits resembling those on single-capsid particles occurred spontaneously, although slowly. These subunits have also been seen following selective removal of VP6 from the inner capsid of virus particles (4). The present data indicate that interactions with other viral proteins or with preformed subviral structures are not required for subunit formation, although such interactions may accelerate the process. The availability of large amounts of purified capsid proteins will now facilitate dissection of the factors that control particle morphogenesis and may ultimately allow self-assembly of virus capsids, as recently accomplished with expressed VP1 of polyomavirus (24). The characteristics of VP6 shown here also extend the known properties of proteins synthesized with the baculovirus expression system. The native conformation of SA11 VP6 reported here is consistent with other studies that show the use and advantages of this system to produce eucaryotic proteins (16, 18, 19, 26, 28).

The possible uses of expressed VP6 to improve current diagnostic tests for rotaviruses are of considerable interest. Antiserum made to unpurified or partially purified VP6 detected common antigenic determinants shared by all group A rotaviruses. An unexpected property of this antiserum (made even to unpurified protein) was its lack of reactivity with mammalian cell proteins. Further evaluation will determine whether this serum will significantly improve sensitivity or specificity of diagnostic tests. However, since VP6 is the major protein detected in commercial diagnostic tests based on either monoclonal or polyclonal antisera, the future production of such reagents by using baculovirus-expressed VP6 (or other rotavirus proteins) might be cost-effective due to the high yields and the ability to produce this protein from infected cells grown in spinner cultures. Expression of the proteins of other rotavirus strains, particularly noncultivatable strains such as some human group A or the non-group A rotaviruses, could be particularly beneficial.

Future efforts will evaluate the use of expressed VP6 as a vaccine, using animal models that can measure passive and active protection against virus challenge. Antisera to expressed VP6 produced in the current study did not neutralize virus in plaque reduction assays. This result agrees with previous reports that monoclonal antibodies to VP6 do not neutralize virus in vitro (13, 22, 29), but it contrasts with other reports that antiserum to gel-purified VP6 possesses low titers of neutralizing antibodies (2, 15, 23). The inability of the expressed VP6 to induce neutralizing antibodies, however, does not rule out a possible role for this protein in inducing protection from infection or disease, as our understanding of the immunologic and nonimmunologic mechanisms that are critical to induce protection from rotavirus infection and disease remains incomplete (8). VP6 might be important in stimulating cell-mediated immune responses

FIG. 6. Assembly of expressed VP6 into structures with capsomerelike morphology. (A) Tubular structures seen in samples of expressed VP6. These structures show hexagonal subunit arrangements typical of the rotavirus inner capsid. (B) VP6 structures labeled with αSGI-gold complex. (C) VP6 structure reacted with αNS29-gold complex. No specific binding occurred. Magnification bars equal 100 nm.

that induce reported heterotypic immunity in a fashion similar to that of the proteins of influenza virus (1, 30, 31). Baculovirus-expressed VP6 may also be a useful component of future subunit vaccines containing additional expressed outer capsid (VP3 and VP7) proteins.

ACKNOWLEDGMENTS

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LITERATURE CITED

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