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Gene Protein Products of SA11 Simian Rotavirus Genome

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When MA104 cells were infected with SA11 rotavirus, 12 protein classes, absent in mock-infected cells, could be distinguished by polyacrylamide gel electrophoresis. At least two of these proteins were glycosylated, and their synthesis could be blocked with tunicamycin. The oligosaccharides of both glycoproteins were cleaved by endo-β-*N*-acetylglucosaminidase H, suggesting that they were residues of the "high-mannose" type. Of the 12 viral polypeptides observed in infected cells, 1 was probably the apoprotein of one of these glycoproteins; 5, including 1 glycoprotein, were structural components of the virions, whereas the other 6, including a second and possibly third glycoprotein, were nonstructural viral proteins. When the 11 double-stranded RNA genome segments of SA11 were translated, after denaturation, in an RNA-dependent cell-free translation system, at least 11 different polypeptides were synthesized. Ten of these polypeptides had electrophoretic migration patterns equal to those of viral proteins observed in tunicamycin-treated infected cells. Nine of the 11 double-stranded RNA genome segments were resolved by polyacrylamide gel electrophoresis and were translated individually. Two were not resolved from each other and therefore were translated together. Correlation of each synthesized polypeptide with an individual RNA segment allowed us to make a probable gene-coding assignment for the different SA11 genome segments.

Tissue culture-adapted simian rotavirus SA11 (17) has become a model system of the rotaviruses, members of the Reoviridae family that cause enteritis in the young of many mammalian species (8, 11). SA11 virions are composed of a genome, consisting of 11 segments of double-stranded (ds) RNA (25), and of a double-layered capsid, consisting of at least five protein classes, three of which compose the inner capsid and two compose the outer capsid (6). Interestingly, one of the outer capsid proteins is glycosylated (18, 26) even though this virus does not have a lipid-containing membrane. A greater number of SA11 structural protein classes (8 to 10) have been previously reported (7, 12, 18, 26, 30), but two of these putative structural polypeptides have been found to be trypsin cleavage products of one of the larger structural polypeptides (6); others, present in small amounts, have not always been observed (6, 7, 12, 18, 26, 30).

SA11 rotavirus contains an endogenous RNA-dependent RNA polymerase which synthesizes mRNA from each of the dsRNA genome segments (18). The number of protein classes synthesized in SA11-infected cells is obscure: Smith et al. (30) have reported only two nonstructural SA11 polypeptides; Mason et al. (18) have designated five of the polypeptides found in infected cells as nonstructural or pretermination products; and Matsuno et al. (19) have reported four nonstructural viral proteins.

In vitro translation of individual denatured dsRNA SA11 genome segments has allowed a probable gene-coding assignment of each of the six larger segments (30). Translation of either denatured SA11 RNA (30) or RNA transcripts synthesized by the endogenous viral RNA-dependent RNA polymerase (18) suggested that processing must occur for the production of some structural polypeptides.

In this paper, we examine the gene protein products of SA11 and report six probable nonstructural polypeptides, two of which are glycosylated, having a "high-mannose"-type carbohydrate moiety. Finally, we make a probable gene-coding assignment for each of the SA11 dsRNA segments, including those smaller segments not reported by Smith et al. (30), by comparing the in vitro and in vivo translation products of SA11 RNA.

MATERIALS AND METHODS

Virus. Simian rotavirus (SA11), obtained originally from H. H. Malherbe, University of Texas, was grown in MA104 cells and purified as previously described (6). In brief, a culture of infected MA104 cells showing 80 to 100% cytopathic effect was extracted with an

equal volume of trifluorotrichloroethane, and the virus in the aqueous phase was subsequently precipitated with 8% (wt/vol) polyethylene glycol (Union Carbide Corp., New York, N.Y.). The precipitate was suspended in approximately 200 μl of TSM buffer (0.01 M Tris-hydrochloride, 0.15 M NaCl, and 0.001 M $MgCl_2 \cdot 6H_2O$, pH 8.2) and then sedimented in a linear sucrose gradient (15 to 45% [wt/vol] TSM) by centrifugation at 19,000 rpm for 145 min in an SW40 rotor of a Beckman ultracentrifuge. After centrifugation, the opalescent (radioactive, when labeled) band of virus was collected. After layering on top of a solution of CsCl in TM buffer (0.01 M Tris-hydrochloride–0.001 M $MgCl_2 \cdot 6H_2O$, pH 8.2) having a density of 1.36 g/cm^3, it was centrifuged to equilibrium at 40,000 rpm for 17 h. After centrifugation, the opalescent band of virus was collected and dialyzed against TSM buffer. To prepare labeled virus, minimal essential medium containing a low concentration of L-methionine (0.3 mg/liter) and 33 μCi of L-[^{35}S]methionine per ml (>500 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was used to replace cold medium 6 h postinfection. At 22 h postinfection, the infected cultures were harvested, and the virus was purified as described above.

Radioactively labeled infected cells. Confluent monolayers of MA104 cells in 25-cm^2 plastic flasks were washed twice with phosphate-buffered saline and infected with SA11 virus. The virus was allowed to adsorb to the cells for 1 h at 37°C; the medium then was replaced by minimal essential medium. Tunicamycin, when used, was added to the medium to give a final concentration of either 2 or 10 μg/ml. At 6 h postinfection, the medium was changed to modified minimal essential medium, which had a lower concentration of L-methionine (0.3 mg/liter) and contained 33 μCi of L-[^{35}S]methionine per ml. After 2 h of incubation, the cells were washed twice with phosphate-buffered saline and disrupted in 200 μl of Laemmli sample buffer by heating for 2 min in boiling water (14). Radioactively labeled glycoproteins were obtained by the same procedure, using modified minimal essential medium which had 250 mg of glucose and 500 μCi of either D-[^{3}H]glucosamine or D-[^{3}H]mannose (New England Nuclear) per liter.

Preparation of RNA and isolation of individual segments. RNA was obtained from purified virus which had been disrupted with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (7). The disrupted virus was treated twice with 1 volume of phenol saturated with TE buffer (0.1 M Tris-hydrochloride–0.001 M EDTA, pH 8.2) and twice with 1 volume of chloroform (10). The RNA in the separated aqueous layer was subsequently precipitated by addition of 0.04 volumes of 5 M NaCl and 2 volumes of ethanol; the solution was kept at −20°C overnight, and the RNA which precipitated was centrifuged at 16,000 × *g* for 30 min. For preparative RNA electrophoresis, polyacrylamide-bisacrylamide (5% [wt/vol], 0.125% [wt/vol]) slab gels were prepared as described (7). Approximately 30 μg of viral RNA was layered onto the gel, and the electrophoresis was carried out at 20 mA/gel. After the RNA bands were stained with ethidium bromide (1 μg/ml of water), they were visualized under a long-wave UV lamp; each section of the gel which contained a band was excised with a razor blade and was finely minced. The RNA in each band was then eluted separately by shaking each minced gel in 3 to 4 ml of TE buffer for 2 to 3 days at room temperature. After elution, the ethidium bromide and monomeric acrylamide which were eluted with the RNA were removed with phenol and isoamyl alcohol as previously described (20), and the RNA was precipitated as above. After centrifugation, the RNA was dissolved in 90% dimethyl sulfoxide to give a final concentration of approximately 80 μg/ml.

In vitro translation. Immediately before translation, the dsRNA in dimethyl sulfoxide was denatured by heating to 50°C for 15 min; the denatured dsRNA was added directly to the translation reaction mixture in an amount sufficient to give a final concentration of 40 to 50 μg/ml, but not to exceed 1.8% dimethyl sulfoxide (20). Rabbit reticulocyte lysate, obtained either by the procedure of Villa-Komaroff et al. (34) or from New England Nuclear, was used as the translation mixture as indicated in the figure legends. The conditions for protein synthesis with the lysate obtained by the method of Villa-Komaroff et al. were as described by Pelham and Jackson (23) with the following modifications: 0.25 mM each of the 19 unlabeled amino acids, 30 μg of micrococcal nuclease per ml (P. L Biochemicals), and 8 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid were used. The conditions for protein synthesis with the commercial translation kit were as specified by the manufacturer.

Immunoprecipitation. Immunoprecipitation was performed essentially as previously described (29), using guinea pig antiserum prepared against purified double-shelled SA11 virus particles. Cells were washed twice with wash buffer (137 mM NaCl, 20 mM Tris-hydrochloride, 0.92 mM $CaCl_2$, and 0.49 mM $MgCl_2$) and then were disrupted with extraction buffer (wash buffer containing 10% glycerol and 1% Nonidet P-40). Cellular debris was removed by centrifugation for 10 min in an Eppendorf microcentrifuge. To each 0.5 ml of supernatant, 10 μl of antirotavirus serum was added followed by 20 μl of a 50% suspension of *Staphylococcus aureus* protein A coupled to Sepharose CL-4B beads (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The mixture was agitated gently for 30 min at 4°C, and the Sepharose beads were then washed three to four times with phosphate-buffered saline.

Endo-β-*N*-acetylglucosaminidase H treatment. The same cellular extract used for immunoprecipitation was used for endoglycosidase H assays. Five microliters of extract in 10 μl of phosphate-citrate buffer (0.15 M, pH 5.0) was incubated for 15 min at 37°C with variable amounts of endoglycosidase H (Miles Laboratories, Inc., Elkhart, Ind.) as indicated in the figure legends.

Polyacrylamide gel electrophoresis of polypeptides. Samples of synthesized polypeptides were analyzed on polyacrylamide-bisacrylamide (either 15%/0.4% or 11%/0.3%, wt/vol, as indicated in the figure legends) slab gels according to the method of Laemmli (14). Proteins were dissociated by treatment with Laemmli sample buffer (14) in a boiling water bath for 2 min. Polypeptides were stacked at 10 mA/gel, and electrophoresis was continued at 20 mA/gel. Gels were subsequently fixed and stained with 30% methanol–10% acetic acid solution containing 0.06% (wt/vol) Coomassie brilliant blue. After destaining in 10% acetic acid, gels were processed for fluorography according to Bonner and Laskey (2).

Molecular weight determinations. Molecular weights

of the small polypeptides were calculated by extrapolation of the linear regression between the observed electrophoretic migrations and the molecular weights reported for SA11 polypeptides by Mason et al. (18).

Peptide mapping. NCVP5 and pNCVP5 were obtained from lysates of cells infected either in the absence or in the presence of 2 μg of tunicamycin per ml of medium, respectively, and labeled with 50 μCi of [^{35}S]methionine per ml of medium from 6 to 8 h after infection. Proteins were separated by gel electrophoresis, and then the radioactive bands corresponding to NCVP5 and pNCVP5 were located and excised (6). Comparison of the products obtained by limited proteolysis according to Cleveland et al. (3) was performed as previously described (6). Tryptic peptide maps were obtained as follows. Trypsin digestion was accomplished in the gel slices containing each protein essentially as described by Morrison and Lodish (21). Briefly, the gel slices were placed in 200 μl of 0.05 M ammonium bicarbonate solution containing 50 μg of trypsin (type XI; Sigma Chemical Co., St. Louis, Mo.). The solution was incubated at 37°C overnight, and then incubation was continued for another 4 h after the addition of 50 μg of fresh trypsin. The gel slices were subsequently removed, and the trypsin-ammonium bicarbonate solution was lyophilized. The resulting peptides were analyzed as described (28); electrophoresis was performed at 400 V for 90 min, using pyridine-acetic acid-water (2:20:180) as the buffer system (28). Ascending chromatography was performed with *n*-butanol–acetic acid–water (3:1:1) (28). The cellulose sheets were processed for fluorography as described (13).

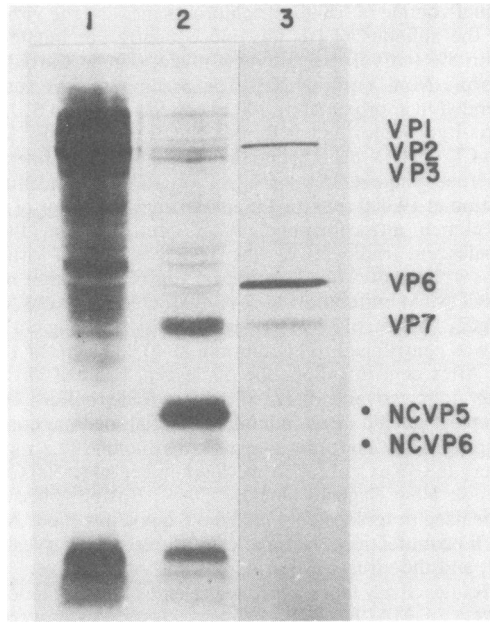

FIG. 2. Fluorogram of polypeptides in SA11-infected MA104 cells pulse-labeled for 30 min with [^{3}H]mannose at 8 h postinfection after analysis on a SDS–11% polyacrylamide gel electrophoresis. Track 1, Mock-infected cells; track 2, infected cells; track 3, [^{35}S]methionine-labeled purified SA11 virus.

RESULTS

Rotavirus SA11 polypeptides in infected cells. When [^{35}S]methionine-labeled SA11-infected cell extracts were analyzed by polyacrylamide gel electrophoresis, 11 protein bands which were absent in mock-infected cells could be distinguished (Fig. 1A). There were, however, at least 12 different putative viral protein classes in infected cells, since NCVP1, which comigrated with VP3, could only be clearly resolved when the electrophoresis was run for a period three times longer (Fig. 1B, track 2). Five of these 12 bands corresponded, according to their electrophoretic mobility, to the structural polypeptides VP1, VP2, VP3, VP6, and VP7 (see also Fig. 4B; the nomenclature originally used by Rodger and Holmes [25] and adopted by Mason et al. [18] will be used). Since VP4, previously reported as a structural polypeptide (25), could not be observed in purified virus (Fig. 1B, track 1), it has been designated NCVP1. The polypeptide with the greatest electrophoretic migration, seen at the bottom of the gel (Fig. 1A), was the unglycosylated precursor of a viral glycoprotein called NCVP5 (see below); henceforth, we designate this polypeptide as pNCVP5 and the other nonstructural polypeptides as NCVP1 through NCVP6, in order of decreasing molecular weight.

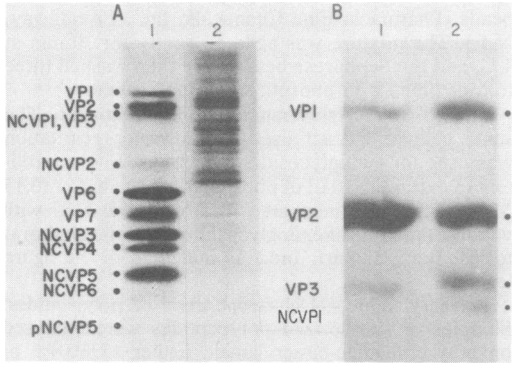

FIG. 1. (A) SDS–11% polyacrylamide gel analysis of the extracts from SA11-infected MA104 cells (track 1) and from mock-infected cells (track 2); both preparations were pulse-labeled for 30 min with [^{35}S]methionine at 8 h postinfection. (B) The band patterns obtained when the extract used in (A) (track 2) and disrupted [^{35}S]methionine-labeled purified virus (track 1) were run for a period three times longer than that in (A). (VP refers to capsid proteins; NCVP refers to noncapsid viral protein; pNCVP refers to precursors of NCVP; and pVP refers to precursors of VP).

Viral glycoproteins and unglycosylated precursors in SA11-infected cells. Three of the 12 polypeptides observed in infected cells were

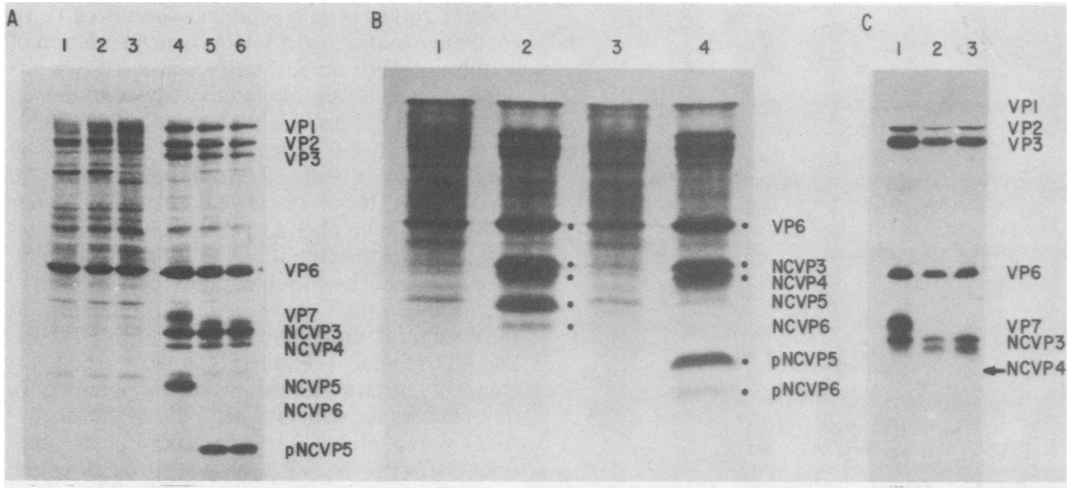

FIG. 3. Effect of tunicamycin in the synthesis of SA11 proteins. (A) SDS–11% polyacrylamide gel analysis of polypeptides from SA11-infected MA104 cells pulse-labeled for 2 h with [^{35}S]methionine at 6 h postinfection. Extracts from mock-infected cells grown with either 10, 2, or 0 μg of tunicamycin per ml are shown in tracks 1, 2, and 3, respectively. Extracts from infected cells grown with either 0, 2, or 10 μg of tunicamycin per ml are shown in tracks 4, 5, and 6, respectively. (B) SDS–15% polyacrylamide slab gel analysis. Extracts from mock-infected (tracks 1 and 3) or from SA11-infected (tracks 2 and 4) cells, grown without (tracks 1 and 2) or with (tracks 3 and 4) tunicamycin (2 μg/ml). (C) Electrophoresis in a SDS–11% polyacrylamide gel after immunoprecipitation of disrupted SA11-infected MA104 cells, labeled as in (A), by antiserum to purified virus. Tunicamycin presence at 0, 2, or 10 μg/ml is shown in tracks 1, 2, and 3, respectively.

radioactively labeled after addition of either [^{3}H]mannose (Fig. 2, track 2) or [^{3}H]glucosamine (not shown). One of these was the structural glycoprotein VP7, in agreement with previous reports; the other two were nonstructural proteins, designated NCVP5 and NCVP6. Tunicamycin, which blocks the glycosylation of many glycoproteins without causing other significant changes in cells (10, 16, 31), inhibited both the incorporation of [^{3}H]mannose in infected cells by more than 90% and the synthesis of the three glycosylated viral proteins VP7, NCVP5, and NCVP6. This allowed the observation of their possible unglycosylated precursors, which have been tentatively designated pVP7 (previously called VP7a [18]), pNCVP5, and pNCVP6 (Fig. 3A, tracks 5 and 6). The latter two polypeptides were better resolved in gels with a higher concentration of polyacrylamide (Fig. 3B, track 4). Polypeptide pVP7, which migrated very close to NCVP3 and NCVP4, could be more clearly observed after immunoprecipitation with antiserum against purified SA11 (Fig. 3C). The presence of NCVP3 in the immunoprecipitate may have been due to mechanical trapping of this polypeptide, since it is present in intracellular particles which also contain most of the structural polypeptides (R. T. Espejo et al., unpublished data). Treatment of the infected-cell extract with endoglycosidase H, an enzyme that hydrolyzes high-mannose chains from glycoproteins (32), produced the same unglycosylated proteins obtained after infection in the presence of tunicamycin (Fig. 4). However, endoglycosidase H also produced an additional protein that seemed to comigrate with NCVP6. This protein, which was not observed in cells infected in the presence of tumicamycin, presumably corresponded to NCVP5 with one of its possible two oligosaccharide residues hydrolyzed by endoglycosidase H, as reported for the glycoprotein G of vesicular stomatitis virus (9). When purified SA11 was treated with endoglycosidase H, VP7 was converted into pVP7 (data not shown).

The precursor-product relationship between pNCVP5 and NCVP5 suggested by use of tunicamycin and by endoglycosidase treatment was further studied by comparison of their products after both partial and complete protease digestion. The comparison of the products obtained after limited proteolysis with chymotrypsin and *S. aureus* V8 protease was difficult to interpret because although the gel patterns obtained with pNCVP5 and NCVP5 were similar, there was not identity between most of the peptides generated from each protein (not shown). However, differences were expected because the carbohydrate residues which alter the mobility of NCVP5 would also alter the mobility of many of the products generated by limited proteolysis. Tryptic peptide analysis of pNCVP5 and NCVP5 showed that the amino acid sequences of these proteins were very similar or identical (Fig. 5); in this case, probably most or all tryptic

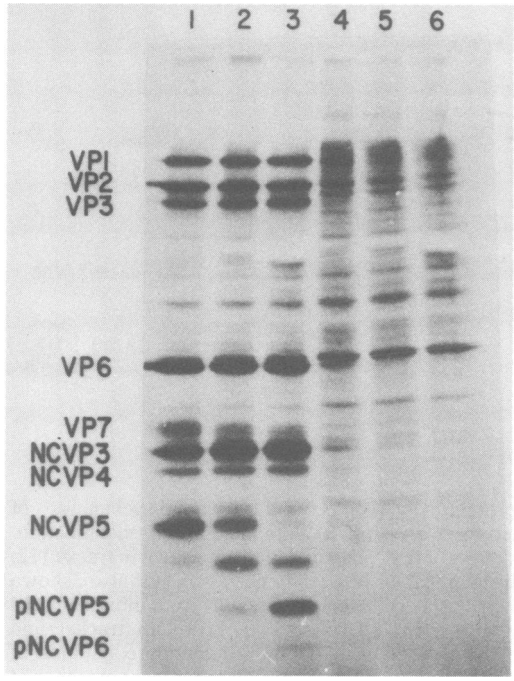

FIG. 4. Effect of endoglycosidase H on SA11 glycoproteins. Fluorogram of a SDS–11% polyacrylamide gel of extracts from SA11-infected MA104 cells treated with 0, 5, or 10 mU of endoglycosidase H (tracks 1, 2, and 3, respectively) and of mock-infected cells treated with 0, 5, or 10 mU of endoglycosidase H (tracks 4, 5, and 6, respectively). Five microliters of extract in 10 μl of phosphate-citrate buffer (0.15 M pH 5.0) was incubated with the indicated amounts of the enzyme for 15 min at 37°C.

methionine-containing peptides did not hold carbohydrate residues because there were no obvious differences between the fingerprints of the glycosylated and unglycosylated proteins.

SA11 rotavirus polypeptides synthesized in vitro from denatured viral RNA. The translation of suitably denatured SA11 RNA in a messenger-dependent cell-free reticulocyte system yielded at least 10 [^{35}S]methionine-labeled polypeptides when the translation products were analyzed by polyacrylamide gel electrophoresis (Fig. 6). There were, however, at least 11 polypeptides since, as shown above, VP3 and NCVP1 comigrated under the conditions used for electrophoresis. Four of these polypeptides corresponded, according to their electrophoretic migration, to the structural viral polypeptides VP1, VP2, VP3, and VP6 (Fig. 6A). Figure 6B shows the comparison of the translation products obtained in different assays with a better yield of the smaller SA11 polypeptides. The nonstructural polypeptides NCVP1, NCVP2, NCVP3, NCVP4, and pNCVP5 were also made in vitro and, hence, they were primary translation products (Fig. 6A, B). As expected, the glycoproteins VP7, NCVP5, and NCVP6 did not appear to be made in the reticulocyte system, because glycosylation does not occur in this system. Figure 6C shows that some of the in vitro-synthesized polypeptides, which were not observed in the infected cells, corresponded to the unglycosylated precursors of the SA11 glycoproteins pNCVP5 and pNCVP6, obtained after infection in the presence of tunicamycin. The fastest-migrating polypeptide synthesized in vitro was not detected in vivo whether tunicamycin was used or not.

Polypeptides from individual SA11 dsRNA segments. SA11 RNA was fractionated by polyacrylamide gel electrophoresis (Fig. 7), and each isolated RNA segment was independently translated after its denaturation. Segments 7 and 8 were exceptions which were translated together because they could not be separated from each

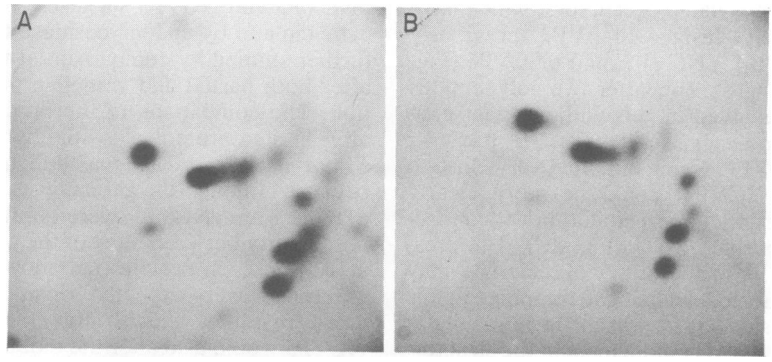

FIG. 5. Tryptic peptide mapping analysis of pNCVP5 and NCVP5. Tryptic digests of [^{35}S]methionine-labeled SA11 polypeptides were obtained and analyzed by two-dimensional separation on thin-layer cellulose plates as described in Materials and Methods. (A) [^{35}S]methionine-labeled peptides of NCVP5; (B) [^{35}S]methionine-labeled peptides of pNCVP5. Electrophoresis was toward the cathode from left to right, and chromatographic development was from the bottom to the top.

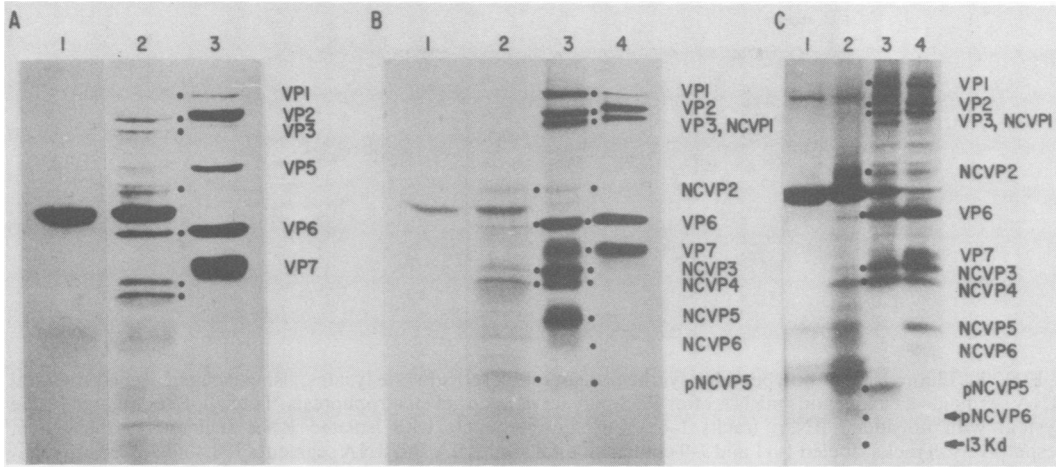

FIG. 6. Comparison between SA11 polypeptides synthesized in reticulocyte lysates programed with denatured SA11 RNA and viral polypeptides synthesized in vivo. (A) In vitro translation products of endogenous mRNA and denatured SA11 RNA (tracks 1 and 2, respectively); purified virus, track 3. (B) In vitro translation products of endogenous mRNA and denatured viral RNA (tracks 1 and 2, respectively); SA11-infected MA104 cells, track 3; purified virus, track 4. (C) In vitro translation products (using commercial reticulocyte lysate) of endogenous mRNA and denatured viral RNA (tracks 1 and 2, respectively); cells infected with SA11 in the presence of tunicamycin (2 μg/ml), track 3; SA11-infected MA104 cells, track 4. In all cases the label was [^{35}S]methionine, and the electrophoresis was performed in an 11% polyacrylamide gel.

other under the conditions used. RNA segment 9, however, was separated from segments 7 and 8 for translation assays from gels run for longer times (not shown). The identification of the [^{35}S]methionine-labeled polypeptides synthesized when these RNA segments were used as exogenous mRNA (Fig. 8) allowed the following gene-coding assignments: RNA segment 1, VP1; 2, VP2; 3, VP3; 4, NCVP1 and a small polypeptide of 13 kilodaltons not found in infected cells; 5, NCVP2; 6, VP6; and 7, 8, and 9, NCVP3, NCVP4, and possibly pVP7. VP6, the product of RNA segment 6, hidden in this figure, is clearly shown in Fig. 9, which also shows the product of isolated segments 7, 8, 9, and 10. There are, presumably, two polypeptides in the position of NCVP3, NCVP3 and pVP7, the unglycosylated polypeptide of VP7; one would be coded by RNA segment 7 or 8, and the other would be coded by RNA segment 9. pNCVP5, the possible apoprotein of NCVP5, is probably coded by segment 10. All attempts to translate the isolated dsRNA segment 11 were unsuccessful; however, pNCVP6, which did not seem to be coded by any other viral RNA segment, is a likely product of this segment.

DISCUSSION

Analysis of the SA11 proteins synthesized in infected cells, or in rabbit reticulocyte lysates upon addition of the denatured dsRNA viral segments, has allowed us to identify the protein products of most of the SA11 genome segments.

VP1, VP2, and VP3. VP1, VP2, and VP3 are structural polypeptides coded by dsRNA segments 1, 2, and 3, respectively, in agreement with Smith et al. (30), who called them I_1, I_2, and I_3, respectively.

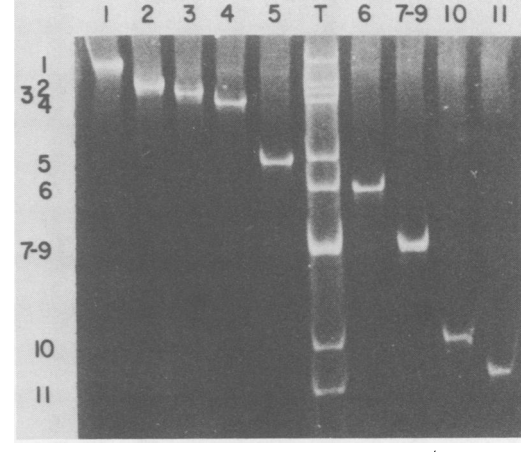

FIG. 7. Electrophoresis in polyacrylamide-bisacrylamide (5% [wt/vol], 0.125% [wt/vol]) of SA11 RNA and the isolated RNA segments. Segments are numbered according to increasing electrophoretic migration. The number of each track corresponds to that of the purified RNA segment. Segments 7, 8, and 9 were not resolved in this gel. T, Total SA11 RNA (3 μg). One-tenth of the amount of each eluted RNA segment which had been obtained from a preparative electrophoresis of 30 μg of SA11 RNA was loaded onto the gel.

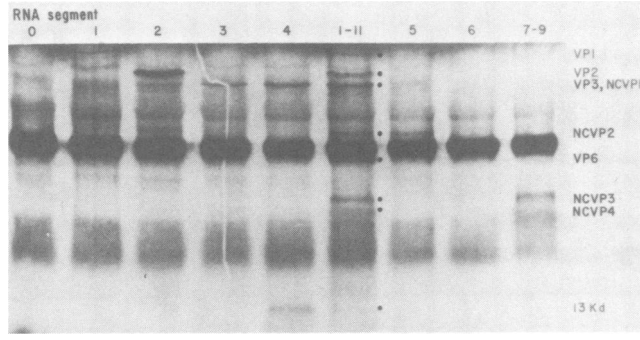

FIG. 8. Fluorogram of polypeptides synthesized in rabbit reticulocyte lysates, using isolated, denatured viral RNA segments as exogenous mRNA after SDS-polyacrylamide gel electrophoresis. Track 0, Reticulocyte lysate system with no added RNA; tracks 1–6, translation products with isolated RNA segments 1 through 6, respectively. Tracks labeled 1–11 and 7–9 contained total viral RNA and RNA segments 7, 8, and 9, respectively.

NCVP1. NCVP1 is a nonstructural polypeptide coded by dsRNA segment 4. From this RNA segment, a small polypeptide with a molecular weight of 13,000 which was not observed in infected cells was also synthesized. As observed with the viral polypeptides obtained in vivo, VP3 and NCVP1 could be resolved from each other only when the electrophoresis was run for a period three times longer (data not shown). NCVP1 or a polypeptide with similar electrophoretic migration has been called I_4 (structural inner capsid polypeptide 4) because it has been occasionally observed in purified virus (30). Although it has been claimed that the protein called I_4 in purified virus comigrates with VP3 and can only occasionally be distinguished from VP3 or I_3 (18, 30), the polypeptide called I_4 by Smith et al. (30) in infected cells migrates noticeably ahead of VP3 and does not appear to correspond to that called I_4 in purified virus (18). Since this polypeptide was observed in infected cells analyzed late after infection, we believe that it more likely corresponds to a possible breakdown product which we also observed late after infection (data not shown).

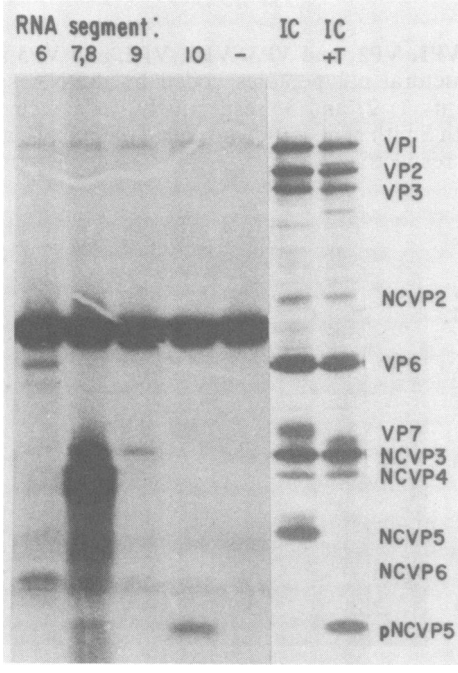

FIG. 9. Fluorogram of polypeptides synthesized in rabbit reticulocyte lysates, using the isolated, denatured viral RNA segments 6 through 10 as exogenous mRNA. Track 6, Denatured RNA segment 6; tracks 7 and 8, denatured RNA segments 7 and 8 translated together; track 9, denatured segment 9; track 10, denatured segment 10; track IC, extract from SA11 virus-infected MA104 cells labeled with [^{35}S]methionine; track IC + T, extract from cells infected in the presence of tunicamycin (2 μg/ml).

NCVP2. The nonstructural viral polypeptide NCVP2 was coded by dsRNA segment 5, in agreement with Smith et al. (30). This polypeptide has been called O_{1A} (30) or VP5a (18) because it was thought to be a possible precursor of VP5, also called O_1. Since VP5 is a trypsin cleavage product of VP3 (6), NCVP2 is most probably a nonstructural polypeptide.

VP6. VP6, a major capsid structural polypeptide, was coded by RNA segment 6, in agreement with a previous report (30).

VP7 and pVP7. VP7 is an outer layer structural glycoprotein with high-mannose-type oligosaccharide residues, according to its sensitivity to endoglycosidase H. pVP7 is the apoprotein generated by treatment with endoglycosidase H of either infected cells or purified virus (data not shown). Infection in the presence of tunicamycin also produced pVP7, which could be precipitated with antiserum prepared against purified virus. A polypeptide with the same electropho-

retic migration as NCVP3 precipitates with this antiserum, and this immunoprecipitable polypeptide might be either NCVP3, present in immature particles of SA11 which contain viral proteins, or another intermediate in the synthesis of VP7. The apoprotein pVP7 is probably coded by segment 7, 8, or 9. As found with other viral glycoproteins (4, 15, 22, 27), the difference in the apparent molecular weight between the putative apoprotein, observed in vitro, which comigrates with NCVP3, and pVP7, observed in vivo, which migrates slightly faster, may be due to the cleavage of a possible leader peptide in the apoprotein synthesized in vivo. The possible precursor-product relationship of VP7 and pVP7, which have also been called O_2 (30) and VP7a (18), has been previously suggested (6, 18).

NCVP3 and NCVP4. The nonstructural polypeptides NCVP3 and NCVP4 were coded by two of the RNA segments 7, 8, and 9. Since RNA segments 7 and 8 coded for two polypeptides having electrophoretic migration equal to that of NCVP3 and NCVP4, and RNA segment 9 coded for one polypeptide with the same migration as NCVP3, these three RNA segments probably code for three polypeptides that can be resolved into only two bands by polyacrylamide gel electrophoresis. These two polypeptides have been also called NS_1 and NS_2, respectively (18, 30).

NCVP5. NCVP5 has the same electrophoretic migration as a protein previously observed in purified SA11 virus and called O_4 (26, 30). However, it is more likely a nonstructural protein because it has not been detected in thoroughly purified virus (6; this study), although it is one of the major viral proteins observed in infected cells. NCVP5 is a glycoprotein with possibly two high-mannose residues because, when treated with endoglycosidase H, an intermediate form was observed as with other glycoprotein with two high-mannose residues (9). Its likely apoprotein precursor, called pNCVP5 (molecular weight, 19,000), observed after infection in the presence of tunicamycin or after endoglycosidase H treatment and also present in small amounts in infected cells, showed a tryptic peptide map nearly identical to that of NCVP5. pNCVP5 and hence NCVP5 are probably coded by RNA segment 10.

NCVP6. NCVP6 is a possible nonstructural glycoprotein, the nonglycosylated precursor of which would be the smaller polypeptide (molecular weight, 16,000) synthesized in tunicamycin-treated infected cells. Since NCVP6 is observed in the same position as the endoglycosidase H partial hydrolysate of NCVP5, its existence is not clearly established.

Most of the polypeptides observed in SA11-infected cells have their homologs in cells infected with rotaviruses isolated from different species. The nonstructural polypeptides NCVP2, NCVP3, NCVP4, and NCVP5 probably correspond to those called O_1, NS_1, NS_2, and O_4 with other rotaviruses (33).

SA11 seems to be an appropriate model for studying not only those rotaviruses important in public health, but also the glycoprotein-containing, nonenveloped viruses. For glycoprotein-containing enveloped viruses, budding is a common process. It is possible that glycoproteins have a role in rotavirus morphogenesis, since immature particles seem to bud into the lumen of the endoplasmic reticulum (1). The glycoproteins of SA11 seem to contain exclusively high-mannose oligosaccharide residues because the same apoproteins obtained in tunicamycin-treated infected cells were generated by treatment with endoglycosidase H. This enzyme, which cleaves the mannose-rich precursor oligosaccharides, will not attack the Golgi-processed oligosaccharides (24). The absence of endoglycosidase H-resistant oligosaccharides in SA11 suggests that complete maturation of virus is obtained in the lumen of the endoplasmic reticulum, in agreement with ultrastructural studies (1). However, endoglycosidase H sensitivity might also be a reflection of the tertiary structure of the protein making the oligosaccharides inaccessible to the Golgi-processing enzymes.

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