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Comparisons of Rotavirus Polypeptides by Limited Proteolysis: Close Similarity of Certain Polypeptides of Different Strains

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The polypeptides of SA11 rotavirus produced in virus-infected cells were analyzed by limited proteolysis, using *Staphylococcus aureus* V8 protease. This clearly distinguished between all the known primary gene products of the virus and allowed relationships between other infected-cell proteins, and between infected-cell and virus structural proteins, to be ascertained. A comparison of the proteolysis cleavage patterns between SA11 rotavirus and the human rotavirus Wa was also performed which demonstrated a marked conservation in the digestion patterns among nonstructural and inner-shell structural proteins, but a marked variation in the digestion patterns among outer-shell structural proteins.

Being of relatively recent discovery and of considerable medical interest as a major cause of infantile gastroenteritis (8, 11, 31), rotaviruses are under intense investigation. They are isometric, containing a genome of 11 distinct segments of double-stranded RNA (10, 20, 24), and are now classified in the family Reoviridae (16). Their capsids are composed of two layers of protein; the inner-shell proteins appear to bear antigenic determinants which are common to all rotaviruses, whereas the outer-shell proteins bear type-specific serological determinants (1, 34).

Although the proteins of purified virions have been well studied (18, 21), virus-specific infected-cell polypeptides have only recently been investigated (15, 17, 28, 29), and the published results are somewhat conflicting, due most probably to the different electrophoretic systems used by the different groups. In particular, Matsuno et al. (17), using a polyacrylamide gel system containing urea, obtained results differing significantly from most others in the number, gel pattern, and molecular weight estimates of infected-cell proteins.

To further characterize the proteins observed in virus-infected cells, we used the biochemical fingerprinting technique of limited proteolysis (3). In this method, proteins are separated on polyacrylamide gels, the individual proteins are cut out and partially digested with a proteolytic enzyme, and the peptides produced are analyzed on another polyacrylamide gel. The patterns of peptides as resolved on the final polyacrylamide gel are characteristic for a particular protein and enzyme. The patterns are also highly reproducible (3).

In this study, we investigated all of the virus-specific infected-cell polypeptides of the simian rotavirus SA11 (14, 21), compared several of these with the corresponding virus structural proteins from purified particles, and also compared them with proteins of the Wa strain of human rotavirus (32).

MATERIALS AND METHODS

Cells and viruses. CV-1 cells were grown in Eagle minimum essential medium containing 10% fetal calf serum and 30 U of gentamicin per ml. SA11 and Wa rotaviruses were originally supplied by H. Malherbe and R. Wyatt, respectively, and the tissue culture-adapted Northern Ireland calf rotavirus was obtained from M. S. McNulty. The viruses were cultivated in MA104 and CV-1 cells as previously described (5). Both viruses have been plaque purified (28, 32).

Production of [^{35}S]methionine-labeled rotavirus polypeptides. Confluent 35-mm plastic petri dishes of CV-1 cells were infected with virus at 10 PFU/ml in the presence of 10 μg of trypsin (1:250, Difco Laboratories) per ml and labeled with [^{35}S]methionine for 1 h at 7.5 h postinfection by following the methods described previously (28). Radiolabeled viruses were produced in CV-1 cells and were purified as previously described (28).

Limited proteolysis analysis. The method of limited proteolysis analysis follows that of Cleveland et al. (3) as modified by Smith et al. (27) and has been described previously (5). Briefly, radiolabeled proteins were separated on preparative sodium dodecyl sulfate (SDS)-polyacrylamide gels (10%), and the bands were excised from the dried gel after they were located by autoradiography. The gel slices were then rehydrated, treated with various amounts of *Staphylococcus aureus* V8 protease (Miles Laboratories, Ltd.) for 30 min at 37°C, and analyzed on 15% polyacrylamide gels. In some experiments, α-chymotrypsin (Worthington Diagnostics) was used instead of V8 protease. V8 pro-

tease cleaves peptide bonds at the COOH-terminal side of aspartic and glutamic acid residues (9).

Polyacrylamide gel electrophoresis. All electrophoreses were performed on vertical slab gels (1.5 mm thick) with the discontinuous buffer system as described by Laemmli (13). Protein samples to be analyzed were treated with Laemmli sample buffer and heated for 5 min at 100°C before being loaded onto the gel. Preparative gels were 10% (wt/vol) acrylamide, whereas analytical gels (for limited proteolysis) were 15% acrylamide (acrylamide/bisacrylamide ratio of 30:0.8). In some gels, urea was added to the running gel (only). Transverse urea gradient gels were prepared by the method of Kew et al. (12). However, the buffer and acrylamide compositions (except for the presence of urea) were the same as those described by Laemmli (13), and a stacking gel (without urea) was present but contained no sample wells, the protein sample being layered right along the top.

RESULTS

SA11 rotavirus polypeptides. Figure 1 shows a typical gel profile of [^{35}S]methionine-labeled SA11 virus-infected cells (track B), and for comparison a profile of uninfected-cell proteins is shown in track A. The nomenclature of virus proteins has been changed from that used previously (28) due to recent advances in the knowledge of rotavirus proteins (6). We have now reverted to the system used initially by Rodger et al. (21) whereby proteins (p) and glycoproteins (gp) are classified according to molecular weight (e.g., p113 is a protein of 113,000 molecular weight). To facilitate interpretation the proteins described in Fig. 1 have also been named (in parentheses) according to the original nomenclature of Thouless (29). The molecular weight estimates used for protein classification have been reported elsewhere (5) and agree closely with those of others (6, 15, 29). We feel that the present nomenclature is necessary until a clearer understanding of rotavirus polypeptides has been established.

Also shown in Fig. 1 (tracks C and D) are the proteins of purified SA11 virus compared with virus-infected cell proteins run on the same slab gel. The preparation of virus was maintained at 0 to 4°C during the purification procedure to minimize proteolytic cleavage (6). It is seen that there is very little of protein p62 in the profile of virion proteins. This preparation shows that there are clearly four high-molecular-weight virus structural proteins (p113, p96, p91, and p84) which have electrophoretic mobilities identical to the virus-specific proteins observed in virus-infected cells (track D). All four proteins have been assigned to individual double-stranded RNA segments (28); however, it now appears that the fourth largest polypeptide (p84), previously thought to be an inner-shell protein (21), is in fact situated on the outer shell of the virus (6).

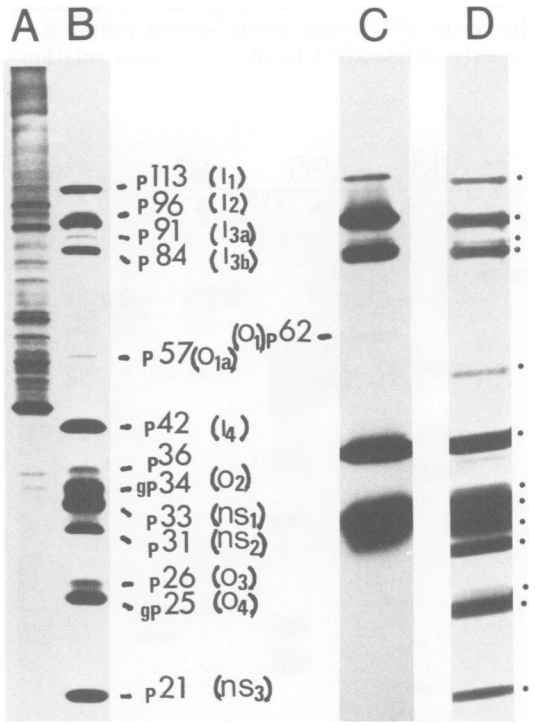

FIG. 1. *SDS-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled (A) uninfected cells, (B) SA11 virus-infected cells, (C) purified SA11 virus, and (D) SA11 virus-infected cells. Tracks A and B were analyzed on a different gel from tracks C and D. Infected-cell and virus proteins were labeled according to their molecular weight (×10^{-3}), and the original nomenclature of M. Thouless for rotavirus polypeptides is indicated in parentheses. The virus-specific proteins shown in track D are indicated by dots and correspond to the proteins labeled in track B. Analyses were on 10 to 20% polyacrylamide gels.*

Limited proteolysis analysis of SA11 proteins. (i) Virus-infected cell proteins. [^{35}S]methionine-labeled proteins from SA11 virus-infected cells were isolated by polyacrylamide gel electrophoresis and treated with *S. aureus* V8 protease, and the peptides were analyzed on gels as described above. Figure 2 shows the limited proteolysis peptide patterns of the high-molecular-weight proteins p113, p96, p91, and p84. It is evident that their gel patterns are all different, which is consistent with previous work in this laboratory which demonstrated that these proteins are all primary gene products, encoded by the four high-molecular-weight double-stranded RNA genome segments (28).

Figure 3A shows the V8 protease patterns of the major outer-shell glycoprotein gp34 (21) and a minor infected-cell protein band p36 (panel A).

The patterns of the major peptides are identical; however, differences occur among minor (i.e., weakly radioactive) bands. The exact relationship between these proteins is as yet unknown. We suspect that p36 is also glycosylated (work in progress; see below).

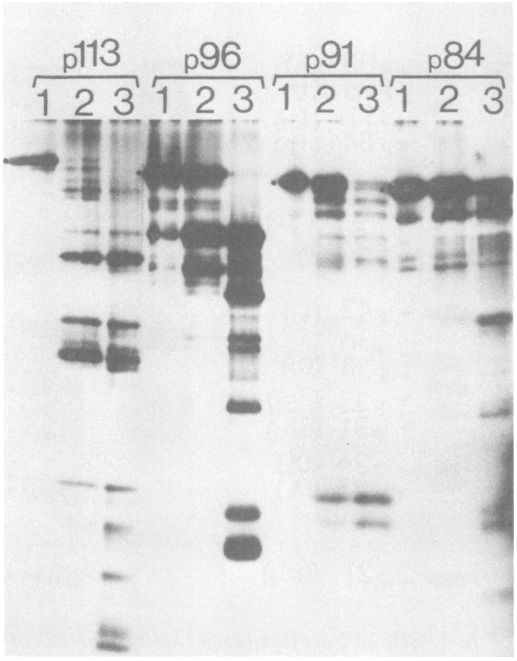

FIG. 2. *Limited proteolysis analyses of SA11 virus-infected cell proteins p113, p96, p91, and p84 digested with (1) 0.05, (2) 0.5, or (3) 10 μg of S. aureus V8 protease for 30 min at 37°C. The uncleaved proteins in this figure and in subsequent proteolysis figures are indicated by dots. Analyses were on 15% polyacrylamide gels.*

Compared in Fig. 3B are the peptide patterns of protein gp34 and the nonstructural proteins p33 and p31. The two nonstructural proteins seem to have qualitatively similar, but not identical, peptide patterns. Also, it appears that peptides of identical mobility to those of gp34 are present in the p33 peptide pattern. This was not apparently due to cross contamination, as preparative gels were electrophoresed long enough to separate the gp34 and p33 bands clearly. (See section iii on the separation of these proteins on urea gels). To further identify possible relationships between the three proteins, a proteolytic comparison was performed with a different enzyme, α-chymotrypsin (Fig. 3C). Although there was less evidence of gp34 peptides in p33, there appeared to be an even greater similarity in the peptide patterns of p33 and p31 when α-chymotrypsin was used.

The V8 protease peptide patterns of proteins p26, gp25, and p21 are shown in Fig. 4. Proteins p26 and gp25 had digestion patterns which were not very complex (i.e., few labeled peptides were produced). Although no peptides were common to both, their overall peptide patterns suggested that they may be related. Protein p21 had a peptide pattern that differed markedly from both p26 and gp25.

When p26 and gp25 were digested with trypsin or α-chymotrypsin (data not shown), it was found in both cases that the peptide patterns were clearly similar (i.e., many peptides were common to both proteins). The corresponding

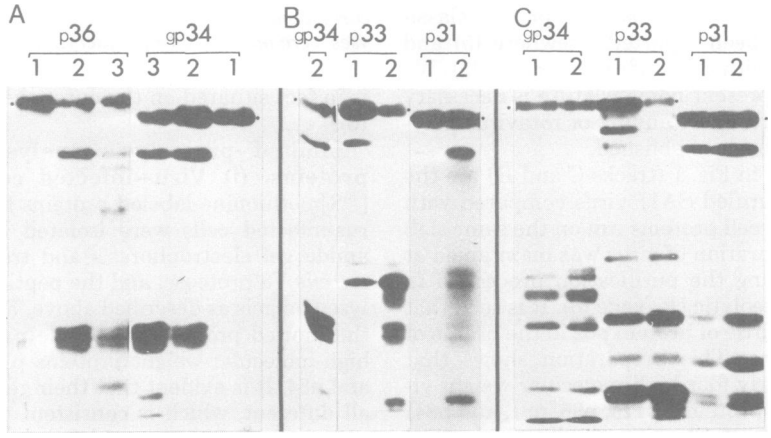

FIG. 3. *Limited proteolysis analyses of SA11 virus-infected cell proteins p36, gp34, p33, and p31. (A) V8 protease digests of p36 and gp34 with (1) 0, (2) 0.5, or (3) 10 μg of enzyme. (B) V8 protease digests of proteins gp34, p33, and p31 with (1) 0.05 or (2) 10 μg of enzyme. (C) α-Chymotrypsin digests of gp34, p33, and p31 with (1) 5 or (2) 10 μg of enzyme. Digestion was at 37°C for 30 min, and peptides were analyzed on 15% polyacrylamide gels.*

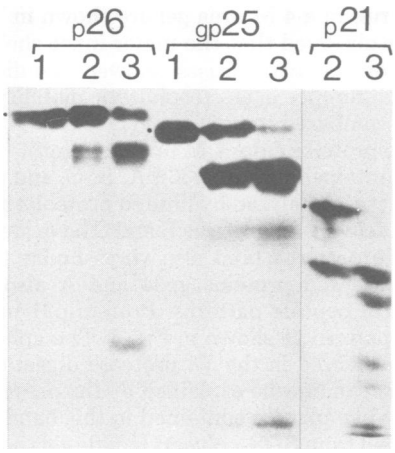

FIG. 4. *Limited proteolysis analyses of SA11 virus-infected cell proteins p26, gp25, and p21, using V8 protease. Proteins were digested for 30 min at 37°C with (1) 0, (2) 0.5, or (3) 10 μg of enzyme and analyzed on 15% polyacrylamide gels.*

proteins of calf rotavirus (p25.5 and gp25), when digested with V8 protease, showed almost identical patterns (Fig. 5).

These results are consistent with proteins gp25 and p21 being primary gene products, encoded by the two smallest double-stranded RNA genome segments (5). The exact relationship between SA11 proteins p26 and gp25 is as yet unknown. We suspect that both are glycosylated, but have not yet been able to demonstrate the presence of carbohydrate in the minor protein p26.

(ii) Comparisons of infected-cell and purified-virus proteins. To investigate the assumed identities of proteins observed in virus-infected cells and in purified virus based on electrophoretic mobilities in SDS-polyacrylamide gels, the limited proteolysis patterns of the major virus structural proteins isolated from both sources were compared. Figure 6 shows the V8 peptide patterns of proteins p96, p42, and gp34 obtained from purified viruses and from infected cells. It is evident that the corresponding infected-cell and virion proteins have identical patterns.

To clarify the relationship among proteins p84, p62, and p57 (Fig. 1), we compared the protease patterns of these polypeptides (Fig. 7). Proteins p84 and p57 were obtained from infected cells, and p62 was obtained from purified virus. It appears that polypeptides p84 and p62 share many peptides, which confirms the conclusion of Espejo et al. (6) that p62 is a tryptic peptide of one of the high-molecular-weight virus proteins; however, the results presented have indicated that it is derived from the fourth largest protein and not the third largest, as suggested by these authors. Protein p57 has a peptide pattern different from the other two proteins,

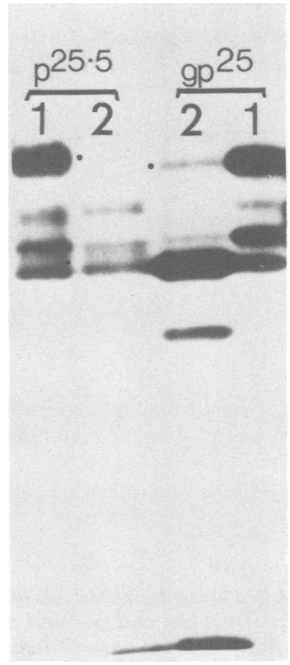

FIG. 5. *Limited proteolysis analyses of Northern Ireland calf rotavirus-infected cell proteins p25.5 and gp25, using V8 protease. Proteins were digested with (1) 0.5 or (2) 10 μg of enzyme and analyzed on 15% polyacrylamide gels.*

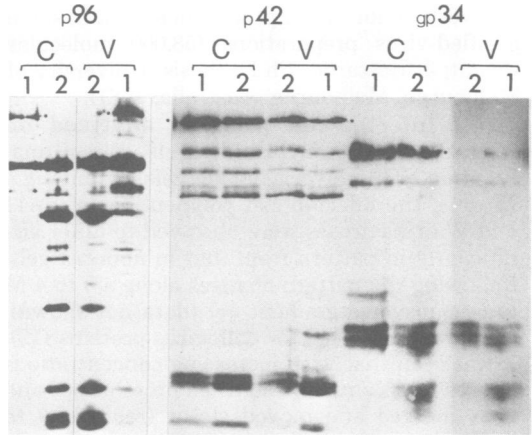

FIG. 6. *Limited proteolysis analyses of SA11 virus-infected cell (C) and purified-virus (V) proteins p96, p42, and gp34, using V8 protease. Proteins were digested with (1) 0.5 or (2) 10 μg of enzyme and analyzed was on 15% polyacrylamide gels.*

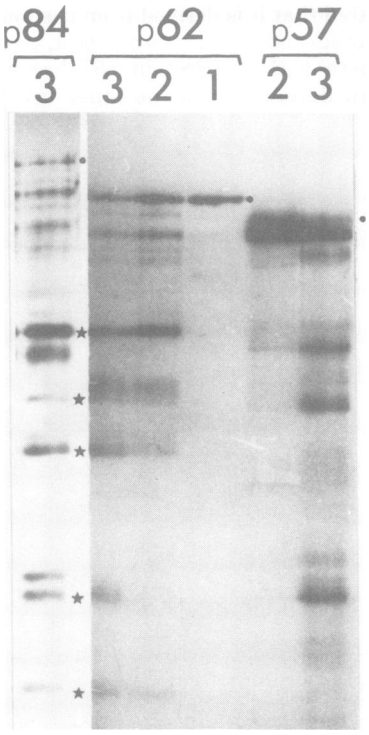

FIG. 7. *Limited proteolysis analyses of SA11 virus-infected-cell proteins p84 and p57 and the purified-virus protein p62. The proteins were digested with (1) 0, (2) 0.5, or (3) 10 μg of V8 protease and analyzed on 15% polyacrylamide gels. Common peptides between p84 and p62 are indicated by stars.*

consistent with the evidence that it is a primary gene product (28). This protein may be related to a very minor virion protein observed in some purified-virus preparations (58,000 molecular weight; J. Bastardo, Ph.D. thesis, University of Melbourne, Melbourne, Australia, 1981).

(iii) Infected-cell proteins analyzed on urea-SDS-polyacrylamide gels. When analyzed on SDS-polyacrylamide gels containing 4 M urea, the infected-cell polypeptides of SA11 and Wa rotaviruses were observed to differ significantly in pattern from that in nonurea gels. Following the pattern changes along a 0 to 4 M urea transverse gradient gel (data not shown), similar to that used for poliovirus proteins (12), it was seen that with increasing concentrations of urea SA11 virus-infected cell proteins p36 and gp34 merged and moved closer (relatively) to the major inner-shell protein p42. Also, the p33 protein band split into three bands: an upper more diffuse band (A) and two closely spaced bands of equal intensity (B and C). There were no dramatic alterations in the behavior of the other proteins. The protein profiles of both viruses run in a 4 M urea gel are shown in Fig. 8. It was observed that the major inner-shell proteins of the two viruses showed significantly more difference in electrophoretic mobility than when compared in nonurea gels (5).

The proteins from 4 M urea gels, gp34 (which also contained protein p36), A, B, C, and p31 of SA11, were analyzed by limited proteolysis (Fig. 9). It is clear that proteins B and C have identical peptide patterns (and also very similar to p33 [Fig. 3]), and proteins gp34 and A also have identical peptide patterns. Protein p31 has the same pattern as shown in Fig. 3. The gp34 peptides observed in the V8 protease digest of p33 (Fig. 3) can now be explained by the presence of a gp34-like protein contained in this band when obtained from SDS-polyacrylamide gels not containing urea.

(iv) Comparison of SA11 and Wa rotavirus polypeptides. The peptide patterns of most of the virus-coded polypeptides of simian rotavirus SA11 and human rotavirus Wa were compared directly. The molecular weights of the proteins of both viruses have been reported previously (5). Figure 10A, B, and C shows the V8

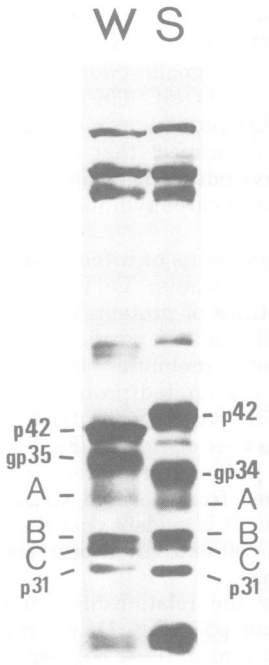

FIG. 8. *Urea-SDS-polyacrylamide gel of Wa rotavirus (W)- and SA11 virus (S)-infected cell proteins labeled with [^{35}S]methionine. The proteins were run on 10% polyacrylamide gels containing 4 M urea. Only the significant proteins were labeled, and some proteins were deliberately run off the end of the gel to separate the p33 band clearly into its three components (A, B, and C).*

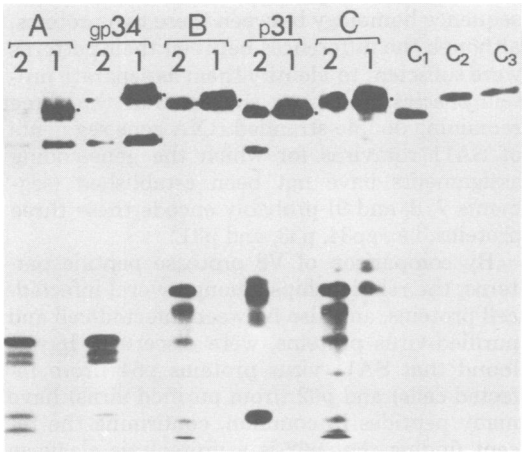

FIG. 9. *Limited proteolysis analyses of SA11 virus-infected cell proteins A, B, C, gp34, and p31 after separation on a 4 M urea-SDS-polyacrylamide gel. Proteins were digested with (1) 0.5 or (2) 10 μg of V8 protease and analyzed on 15% polyacrylamide gels. Controls (proteins not digested with enzyme) are also shown: C_1, C_2, and C_3 indicate the undigested proteins p31, A, and B, respectively.*

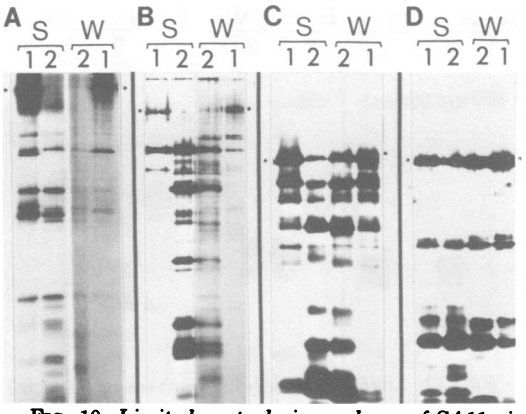

FIG. 10. *Limited proteolysis analyses of SA11 virus- and Wa virus-infected cell proteins. SA11 virus (S) inner-shell proteins p113, p96, and p42 were compared with the corresponding Wa virus (W) proteins (p113, p98, and p42, respectively). (A) V8 protease digests of SA11 virus protein p113 and Wa virus protein p113 with (1) 0.5 or (2) 10 μg of enzyme. (B) V8 protease digests of p96 (SA11) and p98 (Wa) with (1) 0.5 or (2) 10 μg of enzyme. (C) V8 protease digests of protein p42 of both viruses with (1) 0.5 or (2) 10 μg of enzyme. (D) α-Chymotrypsin digests of the p42 protein of both viruses with (1) 5 or (2) 10 μg of enzyme. Analyses were on 15% polyacrylamide gel.*

peptide patterns of the major inner-shell structural proteins (p113, p96, and p42) of SA11 virus compared with the corresponding Wa virus proteins. Apart from minor differences, the peptide patterns were remarkably similar for each of the corresponding proteins. As a further test, the major inner-shell protein of each virus (p42) was analyzed with α-chymotrypsin instead of V8 protease. The result (Fig. 10D) confirmed that indeed the two proteins are very similar.

The major outer-shell structural proteins were then compared. Figure 11A and B shows the V8 protease patterns of polypeptides p84 and gp34 of SA11 virus compared with the corresponding Wa virus proteins, p84 and gp35. Unlike the inner-shell proteins, the corresponding outer-shell proteins of the two viruses differed markedly. Two minor SA11 virus proteins were also analyzed, gp25 and p57 (Fig. 11C and D). Protein gp25 is a major infected-cell protein, but appears to be a very minor protein in purified virus (28, 29), and p57 is a minor infected-cell polypeptide which may be present, also in a very small quantity, in purified virus. Protein p57 was previously thought to be related to an outer-shell protein (29); however, its status is now uncertain (6; this study). Figure 11D demonstrates that proteins p57 (SA11 virus) and p55 (Wa virus) differ significantly in their peptide patterns, especially in their high-molecular-weight peptides. In contrast (Fig. 11C), proteins gp25 (SA11 virus) and gp26,25 (Wa virus) have peptide patterns which appear to be quite similar, most

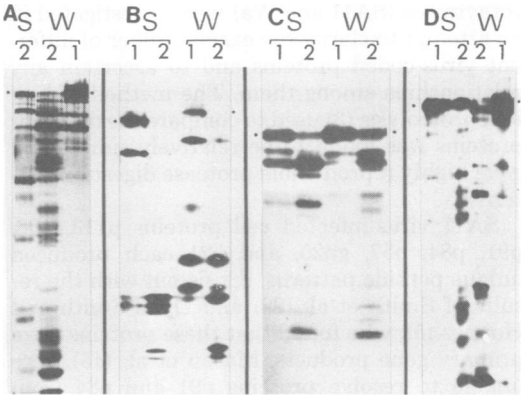

FIG. 11. *Limited proteolysis analyses of SA11 (S) and Wa (W) virus outer-shell proteins. The proteins were digested with (1) 0.5 or (2) 10 μg of V8 protease. (A) Protein p84 of both virus. (B) Proteins gp34 (SA11 virus) and gp35 (Wa virus). (C) Proteins gp25 (SA11 virus) and gp26,25 (Wa virus). (D) Proteins p57 (SA11 virus) and p55 (Wa virus). Analyses were on 15% polyacrylamide gels.*

notably in their high-molecular-weight peptides.

Finally, the SA11 virus nonstructural proteins p33, p31, and p21 were compared with the corresponding Wa virus proteins (Fig. 12). It is apparent that, as with the inner-shell proteins,

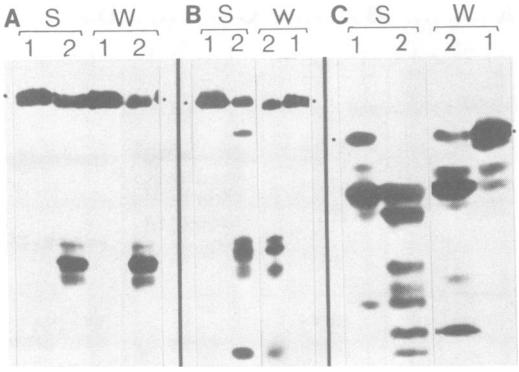

FIG. 12. *Limited proteolysis analyses of SA11 (S) and Wa (W) virus nonstructural proteins, digested with (1) 0.5 or (2) 10 μg of V8 protease. (A) Protein p33 of both viruses. (B) Protein p31 of both viruses. (C) Proteins p21 (SA11 virus) and p22,21 (Wa virus). The p33 proteins were purified of outer-shell protein bands by separation on 4 M urea-SDS-polyacrylamide gels. Peptides were analyzed on 15% polyacrylamide gels.*

the corresponding nonstructural proteins of the two viruses have peptide patterns which are remarkably similar.

DISCUSSION

In this study, the proteins of two different rotaviruses (SA11 and Wa) were investigated in an attempt to clarify the exact number of different virus-coded proteins and to ascertain any relationships among them. The method of limited proteolysis (3) used to compare the different proteins was found to be relatively simple and gave highly reproducible protease digestion patterns.

SA11 virus-infected cell proteins p113, p96, p91, p84, p57, gp25, and p21 each produced unique peptide patterns, consistent with the results of Smith et al. (28) and Dyall-Smith and Holmes (5), who found that these proteins were primary gene products. Mason et al. (15) were unable to resolve proteins p91 and p84 from infected cells or among proteins synthesized in vitro from SA11 virus mRNA, and they thus considered that there were only three primary gene products in the high-molecular-weight region instead of four.

The other main proteins observed in SA11 virus-infected cells (gp34, p33, and p31) were also found to have unique peptide patterns when V8 protease was used, although some peptides of p33 and p31 were similar. Digestion of the latter two proteins with a different enzyme, α-chymotrypsin, also produced patterns with several peptides common to both. This indicates that there is a significant degree of amino acid sequence homology between these two proteins, although the differences between their patterns were sufficient to identify them as separate protein species. Thus, we suggest that the three remaining double-stranded RNA gene segments of SA11 rotavirus for which the gene-coding assignments have not been established (segments 7, 8, and 9) probably encode these three proteins, i.e., gp34, p33, and p31.

By comparison of V8 protease peptide patterns, the relationships among several infected-cell proteins, and also between infected-cell and purified-virus proteins, were discerned. It was found that SA11 virus proteins p84 (from infected cells) and p62 (from purified virus) have many peptides in common, confirming the recent finding that p62 is a proteolytic cleavage fragment of a high-molecular-weight structural protein (6). Infected-cell polypeptides p36 and A were found in minor quantities in infected cells, and their identity was unknown. Limited proteolysis analysis proved that they were related to the major outer-shell protein gp34; however, the exact relationships among these three proteins (p36, gp34, and A) is as yet unknown. Similarly, the exact relationship between proteins p26 and gp25 (SA11) from infected cells is still uncertain, although the apparent similarities in the pattern of digestion with two different proteases strongly suggest that they are related. Confirmatory evidence for this was obtained by using the corresponding calf rotavirus proteins (p25.5 and gp25), which gave almost identical peptide patterns. The difference in electrophoretic mobility between SA11 virus proteins p26 and gp25 (and similarly for the calf rotavirus proteins p25.5 and gp25) may be due to a difference in processing of carbohydrate chains. It is now unclear whether the SA11 virus protein p26 (previously called O_3 [29]) observed in infected cells is present in purified virus, as recent evidence (6) indicates that the protein in this region may be the smaller tryptic peptide of p84.

By a comparison of SA11 virus-infected cells and purified-virus proteins, it was demonstrated that the three major structural polypeptides (p96, p42, and gp34) were indeed identical to the infected-cell proteins having the same apparent molecular weights on SDS-polyacrylamide gels. This confirms the previous assumption of identity for these proteins based upon electrophoretic mobility and also confirms the identities of other minor proteins (e.g., p113 and p91).

Urea was found to affect the relative mobilities of infected-cell proteins in SDS-polyacrylamide gels. It was observed that 4 M urea separated the p33 band into three components (A, B, and C) which were shown to be two forms of

p33 (B and C) and a protein related to gp34 (A). This explains the presence of gp34 peptides in the V8 protease cleavage pattern of p33 isolated from polyacrylamide gels containing only SDS. Protein bands B and C may be analogous to the nucleocapsid-associated protein of vesicular stomatitis virus, which also splits into two bands in polyacrylamide gels containing urea; this effect has been shown to be due to a difference in phosphorylation (2, 4). The urea-SDS-polyacrylamide gel system used by Matsuno et al. (17), who analyzed SA11 and calf rotavirus-infected cell polypeptides, did not appear to resolve proteins clearly compared with the published pictures of others who used polyacrylamide gel systems not containing urea (28, 29). It also seems probable that they have missed the small nonstructural protein p21 (SA11 virus) which may have run off the end of their gels. Protein $NCVP_3$ in the nomenclature of Matsuno et al. probably corresponds to gp25 in our nomenclature.

The most interesting results were obtained when the proteins of two different rotaviruses (SA11 and Wa) were compared. Comparisons by limited proteolysis analysis of four inner-shell structural proteins and three nonstructural proteins demonstrated marked similarities in the corresponding proteins of these viruses. When the major inner-shell virus protein (p42 for both viruses) was analyzed further with α-chymotrypsin instead of V8 protease, the peptide patterns produced by this enzyme were again very similar for the two viruses. This degree of similarity was surprising, since the two viruses used, SA11 and Wa, were isolated on different continents (Africa and North America, respectively) at widely spaced times (1958 and 1979) and from different animals (monkeys and humans). However, the results are consistent with the fact that, with perhaps a few exceptions (23), rotaviruses from all animal species are antigenically very similar (30, 33) and that this cross-reactivity is thought to reside on inner-shell proteins (1, 30).

In contrast to these results, when SA11 virus outer-shell proteins p84 and gp34 were compared with the corresponding Wa virus proteins (p84 and gp35), the peptide patterns were quite different. This is consistent with the notion that virion surface proteins are under greater selective pressure and are more likely to differ antigenically among different virus isolates than are internal and nonstructural proteins (25). It is also consistent with other studies which have shown that the outer-shell proteins of rotaviruses are involved with serological type specificity (1, 34). The results obtained are similar to those found with vesicular stomatitis virus proteins, where it was observed that the major antigenic glycoprotein G, a surface protein, showed much more variation in limited proteolysis patterns than did the internal virus proteins, even when different isolates of the same serotype were analyzed (2). Similarly, in a recent study of the proteins of different serotypes of poliovirus, it was observed that corresponding capsid polypeptides varied considerably in their peptide patterns (22). It was also found that the corresponding noncapsid proteins had remarkably similar peptide patterns, consistent with the results presented in this study.

Protein gp25 of SA11 virus is found in large quantity in virus-infected cells, but is difficult to demonstrate in purified virus (28, 29). The V8 protease digestion patterns of gp25 and the corresponding Wa virus protein (gp26,25) were quite similar, in contrast to the results obtained with the other outer-shell polypeptides. It may be that, although this protein is thought to carry type-specific antigenic determinants demonstrable by enzyme-linked immunosorbent assay (5, 19), it is not involved in virus neutralization (as suggested by the results of Greenberg et al. [7]) and is therefore not under the same selective pressure.

It will be interesting to determine the level of nucleic acid homology between these and other rotaviruses to see whether the similarities and differences observed by limited proteolysis of the proteins are simple reflections of the base sequences. Cross-hybridization studies of the individual genome segments will also be of great interest epidemiologically, and it is hoped that as much information on the important genome segments will be obtainable as in recent studies on influenza virus (25, 26).

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ADDENDUM IN PROOF

After this work was submitted, a publication by McCrae and Faulkner-Valle (J. Virol. **39**:490–496, 1981) appeared. These authors performed pulse-chase analyses on rotavirus-infected cell proteins and found that precursor-product relationships appeared to exist among several proteins. Their results support our findings that proteins p36 and gp34 and p26 and gp25, of SA11 virus are related.

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