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Characterization of Vesicular Stomatitis Virus Mutants by Partial Proteolysis

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Structural proteins of temperature-sensitive (*ts*) mutants of vesicular stomatitis virus, Indiana serotype, were compared with those of wild-type and revertant virions by electrophoresis on polyacrylamide gels of partial digests with *Staphylococcus aureus* V8 protease. Mutants of complementation groups III (*ts*G31 and *ts*G33), II (*ts*G22), and IV (*ts*G41) differed from the wild-type virion in peptide profiles of their M, NS, and N proteins, respectively. The differences were only detectable over a narrow range of enzyme-substrate ratios and were due to peptides transiently generated during incomplete digestion. Proteins of revertants of *ts*G31, *ts*G22, and *ts*G41 exhibited the wild-type virion peptide pattern, indicating that reversion had restored their original conformation. However, in the case of *ts*G22, the NS peptide profile reverted to the wild-type phenotype only partially, suggesting that a silent mutation might have taken place during either the original chemical mutagenesis or the following repeated laboratory passages. The apparent alteration in protein conformation and its restoration upon reversion of the mutants indicated that the lesions of groups III and IV were located in the M and N proteins, respectively. Moreover, for the first time, the site of mutation of group II could be positively identified as the NS protein cistron.

Temperature-sensitive (*ts*) mutants of vesicular stomatitis virus (VSV), Indiana serotype, have been isolated in several laboratories (8, 11, 22, 25). Based on complementation experiments, the mutants were classified into several groups. Chemical mutagenesis usually generated four complementation groups (11, 22), whereas the naturally occurring mutants were placed into five such groups (8). The existence of five complementation groups was consistent with the number of structural virion proteins: L, G, N, NS, and M (31). More recently, the possible existence of a sixth complementation group has been raised (25), but so far has not been independently confirmed. Complementation between mutants obtained from different isolates and cell lines proved to be feasible, making a general classification available (7, 9, 23).

Mutants obtained with the highest frequency belong to complementation group I. Members of this group were shown to be defective in primary transcription when tested in vivo (30) or in vitro (29). Reconstitution experiments established that the thermosensitivity resided in the large (L) protein (12). Mutants of group IV were also found to be thermolabile in primary transcription when tested in vitro (21), but not in vivo (30). This was attributed to a defective template, indicating that either the nucleocapsid (N) or the nonstructural (NS) protein might be the site of mutation (21). Heteroduplex mapping demonstrated that the genome of a group IV mutant (*ts*G41) had an altered N protein cistron (10). This conclusion was consistent with earlier reconstitution experiments which indicated that neither wild-type L nor NS protein could restore the in vitro defective transcriptase activity of the group IV mutant *ts*W16B (12).

The glycoprotein (G) of VSV was implicated as the site of lesion in group V mutants. Some of these mutants were complemented at nonpermissive temperatures by phenotypic mixing with avian myeloblastosis virus, yielding VSV with the host range and interference specificity of the avian virus. This indicated that the particle carried the avian virus glycoprotein (33). It has also been reported recently that at nonpermissive temperatures the group V mutant *ts*O45 generated inactive virions devoid of glycoprotein (26).

Lafay (16) has shown that the matrix (M) protein, synthesized at nonpermissive temperature by a mutant of group III, was not incorporated into the virion after transfer to permissive conditions. Based on this result and a previous observation that less radioactive label is incorporated into the M protein at nonpermissive than at permissive temperature in infections with the group III mutant *ts*O23 (15), the author concluded that the M protein was defective in mutants of this group. However, it should be noted that no such differences in radioactive labeling of the M protein could be observed with

the corresponding Glasgow mutants (32). On the other hand, the demonstration that the M proteins of Massachusetts group III mutants were degraded in vivo three- to fourfold faster than wild-type virion M protein at nonpermissive temperature (13) may explain the differential labeling observed by Lafay. This rapid degradation could not be fully rescued by coinfection with wild-type virions and thus seems to be due to a defect in the M protein itself. Moreover, the rapid degradation of the L protein and N protein of group I and IV mutants, respectively, and the delay in the maturation of the G protein of the group V mutants were consonant with the assignments discussed before (13). However, no consistent phenotype was observed with group II mutants in these or any other studies. Although through a process of elimination the NS protein becomes a plausible candidate for the group II lesion, an N protein defect was suggested from measurements of nucleocapsid stability and pH dependence of its sedimentation coefficient (6).

In addition to the ambiguity of the group II defect, it should also be emphasized that none of the assignments was based on direct measurements of changes in the primary structure of the putative defective proteins. Short of sequence determinations, the currently available techniques may not detect single amino acid replacements, especially if the pertinent amino acid residues do not contain highly reactive groups. However, since the change in primary structure leads to nonfunctional proteins at high temperatures, it is plausible to assume that important conformational changes have occurred in the mutated proteins. A technique, sensitive to minor changes in protein conformation, has been developed and is based on incomplete enzymatic digestions with *Staphylococcus aureus* V8 protease in conjunction with discontinuous polyacrylamide gel electrophoresis of the nascent peptides (3). This method has been shown to be useful in the determination of precursor-product relationships (3), in the analysis of conserved peptides in VSV proteins of different origins (2), and more recently in the determination of the defect in the E1 mutant of the VSV New Jersey serotype (18). We now report its application for determining the defective gene products of certain VSV Indiana serotype *ts* mutants (22). Reproducible differences from wild-type virion in the NS, M, and N proteins of mutants in groups II, III, and IV, respectively, could be demonstrated. In each case, the peptide profile of only one of the four proteins which were examined (NS, M, N and G) differed from that of the corresponding wild-type protein. In revertants, the profiles of the proteins which carried the lesion before reversion became indistinguishable from those of the wild-type virion.

MATERIALS AND METHODS

Cells and media. For large-scale viral preparations, baby hamster kidney cells (BHK-21, clone 13) (19) were grown as monolayers in 32-ounce (ca. 0.946-liter) glass prescription bottles (Kerr Glass Manufacturing Co., Lancaster, Pa.) or disposable plastic tissue culture flasks (150 cm^2) (Corning Glass Works, Corning, N.Y.). For viral plaque assays and clonal purification, BHK-21 cells were grown in 60-mm plastic petri plates (Falcon Plastics, Oxnard, Calif.). Media and growth conditions were as described elsewhere (17, 22, 27).

Viruses. Wild-type and *ts* mutants of VSV, Indiana serotype (Glasgow collection), were kindly provided by C. R. Pringle (22, 23) except for the *ts*G33 mutant, which was obtained from H. F. Lodish. Viral inocula were prepared by a fivefold clonal selection followed by enrichment (28). In the case of *ts* mutants, plaque assays at 31 and 39°C were performed to determine whether the final isolates were temperature sensitive. Revertants of the pertinent *ts* mutants were obtained by picking large plaques obtained at 39°C from the final isolates. The content of the plaques was then enriched by one passage and tested for temperature sensitivity. Progeny with a wild-type phenotype was assumed to have resulted from a reversion.

After enrichment, the supernatant medium was first clarified by centrifugation for 30 min at 4,000 × *g*, and then the virion progeny was pelleted by ultracentrifugation at 54,000 × *g* for 2 h. The pellets were resuspended overnight at 4°C in a small volume of 3E buffer (0.12 M Tris-acetate [pH 7.2], 0.06 M sodium acetate, 0.003 M EDTA), then layered onto a linear 5 to 40% (wt/vol) sucrose gradient and centrifuged in a Beckman SW 25.1 rotor at 51,500 × *g* for 90 min. The viral zone was removed with a Pasteur pipette, diluted threefold with 3E buffer, and centrifuged in a Beckman R 40 rotor at 81,000 × *g* for 45 min. The resulting viral pellet was resuspended overnight in the appropriate buffer and then stored in small aliquots at −70°C.

When ^{35}S-labeled viral proteins were required, 20 ml of methionine-free Glasgow minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) was added to each 150-cm^2 culture flask after infection and incubated 3 h before adding [^{35}S]methionine (840 to 1,200 Ci/mmol, Amersham Corp., Arlington Heights, Ill.) to a final concentration of 5 μCi/ml. Radiolabeled NS protein was prepared by incubation of infected tissue culture flasks with ^{32}P-containing media (^{32}Pi; New England Nuclear, Boston, Mass.) at a final concentration of 50 μCi/ml. Virion progeny was purified in the same manner as described above.

Purification of viral protein by polyacrylamide gel electrophoresis. For preparative isolation of VSV proteins, slab gels (10 by 10 by 0.3 cm) containing 8% acrylamide, 0.21% *N,N*-methylenebisacrylamide (BIS) (Bio-Rad Laboratories, Richmond, Calif.), 0.1% sodium dodecyl sulfate (SDS), and 1 M

urea in 0.1 M sodium phosphate (pH 7.0), were polymerized 1 h by the addition of 0.05% (vol/vol, final concentration) *N,N,N',N'*-tetramethylethylenediamine (TEMED) (Eastman Kodak, Rochester, N.Y.) and 0.075% (wt/vol, final concentration) ammonium persulfate (Bio-Rad Laboratories). Virus suspended in 0.1 M sodium phosphate, pH 7.0, was disrupted by addition of SDS and dithiothreitol (Sigma Chemical Co., St. Louis, Mo.) to final concentrations of 1.0% (wt/vol) and 0.1% (wt/vol), respectively, and the solution was heated at 100°C for 2 min. The samples were then made 10% (vol/vol) in glycerol, applied to wells in the 8% slab gel, and electrophoresed for 20 h at 25 V. Protein bands were localized by staining for 30 min in 0.01% Coomassie blue in 50% methanol–10% acetic acid, followed by destaining for 1 h in 50% methanol–10% acetic acid.

For analytical work, SDS-discontinuous slab gel electrophoresis as described by Laemmli (14) was used with the following modifications. For analysis of the purity of viral proteins isolated by preparative gel electrophoresis, the stacking gel was 1.5 cm long and the running gel was approximately 10 cm long; the running gel contained 10% acrylamide with an acrylamide-to-BIS ratio (wt/wt) of 37.5:1. However, for analysis of limited proteolytic products (see following section), the length of the stacking gel was increased to 3.5 cm, and the running gel was approximately 8 cm long and contained 15% acrylamide; the acrylamide/BIS ratio (wt/wt) was maintained at 37.5:1. Electrophoresis was at 20 mA constant current until the bromophenol blue tracking dye was within 1 cm of the bottom of the gel (5 to 6 h).

Peptide mapping by limited proteolysis. ^{35}S-labeled viral proteins of various isolates of VSV were purified by 8% polyacrylamide gel electrophoresis on slab gels. Protein bands of interest were localized by a short staining with Coomassie blue (see above). The bands were excised from the gel, equilibrated for 30 min in 10 ml of a buffer containing 0.125 M Tris-hydrochloride (pH 6.8), 0.1% SDS, and 1 mM EDTA and then stored at −20°C until needed. Samples of these bands were re-electrophoresed on analytical discontinuous slab gels with marker proteins to confirm integrity and purity.

Limited proteolysis of the proteins in gel slices was conducted as described by Cleveland et al. (3). The slice containing the protein was placed in a sample well of a discontinuous polyacrylamide slab gel that contained a 3.5-cm stacking gel. The sample was then overlaid with a solution of *S. aureus* V8 protease (Miles Laboratories, Elkhart, Ind.) in 0.125 M Tris-hydrochloride (pH 6.8), 0.1% SDS, 1 mM EDTA, and 10% glycerol. The sample was electrophoresed at 20 mA constant current at room temperature until the bromophenol blue tracking dye was within 1 cm of the bottom of the gel, approximately 5 to 6 h. The slab gel was fixed for 15 min in 10% acetic acid–5% glycerol, dried in a gel drier (Bio-Rad Laboratories), and then autoradiographed for 36 to 72 h with Kodak XR5 X-Omat R film (George Brady and Co., Skokie, Ill.).

RESULTS

Plaque assay of *ts* mutants, wild-type virions, and revertants. The *ts* phenotypes of isolates from the Glasgow collection and of the revertants obtained in our laboratory were tested by plaque assays at 31 and at 39°C. The results are shown in Table 1. The stability of the *ts* mutants was demonstrated by the high ratios of log PFU 31°C/PFU 39°C which were as high as seven orders of magnitude and exceeded three orders of magnitude in the most leaky mutants of group III (Table 1, fourth column).

Preparative isolation of ^{35}S-labeled virion proteins. Batches of five 32-ounce tissue culture flasks, each containing 5×10^7 BHK cells, were infected with 10 PFU per cell of the appropriate virus isolate. The viruses were grown in the presence of [^{35}S]methionine, purified, and dissolved in 100 μl of denaturing buffer (see Materials and Methods). The separation of the individual virion proteins obtained by electrophoresis on 8% polyacrylamide gels with a total of 1×10^6 to 2×10^6 cpm (approximately 100 to 200 μg of protein) is shown in Fig. 1. The two bands corresponding to the NS protein are probably due to different degrees of phosphorylation (5). The satellite band in front of the M protein probably corresponds to the Ms fragment of the M protein, observed by other investigators (2). The bands corresponding to G, N, and M proteins were excised, eluted, and re-electrophoresed on analytical 10% polyacrylamide gels to examine their integrity after the purification procedure. Gel profiles of purified proteins are shown in Fig. 2, together with a profile of virion marker proteins. The data indicated that the individual proteins were essentially unchanged by this purification process.

Comparison of partial proteolytic products of group III mutants and their rever-

TABLE 1. *Plaque assay of the VSV-Indiana wild type, ts mutants, and revertants at permissive and nonpermissive temperatures*[a]

Virus isolate	PFU/ml		Log PFU (31°C) / PFU (39°C)
	31°C	39°C	
Wild type (Glasgow)	3.5×10^8	3.6×10^8	0.0
*ts*G22	3.5×10^8	6.0×10^3	4.8
Revertant of *ts*G22	1.2×10^7	1.0×10^7	0.0
*ts*G31	2.3×10^7	5.5×10^3	3.6
Revertant of *ts*G31	2.9×10^6	3.1×10^6	0.0
*ts*G33	5.0×10^7	9.0×10^3	3.7
*ts*G41	1.2×10^8	$<10^2$	>7.1
Revertant of *ts*G41	5.0×10^7	4.0×10^7	0.0

[a] The virions were cloned and purified as described in Materials and Methods. Plaque assays were carried out on confluent monolayers on BHK-21 cells in 60-mm plastic culture plates.

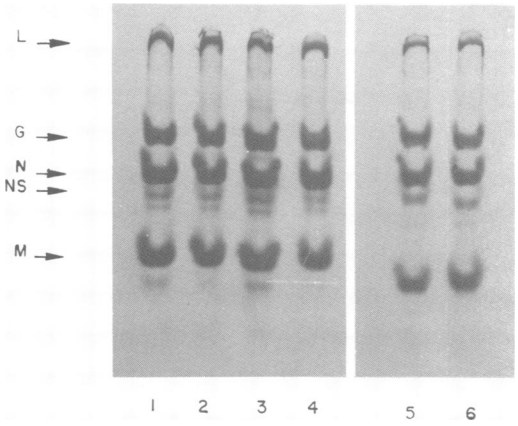

FIG. 1. *Preparative separation of VSV proteins from six different isolates by SDS-polyacrylamide gel electrophoresis. Each VSV isolate was purified from 2 × 10⁸ BHK cells which were incubated in the presence of high specific activity [³⁵S]methionine. The purified virions (see Materials and Methods) were dissolved in sample buffer containing 1% SDS and 1% dithiothreitol, heated for 2 min at 100°C, made 5% (vol/vol) in glycerol, and electrophoresed in an 8% polyacrylamide gel for 20 h at 25 V. Each sample contained approximately 1.0 × 10⁶ to 2.0 × 10⁶ cpm (100 to 200 μg of protein). The proteins were visualized by a 30-min staining with 0.1% Coomassie blue. Lane 1, VSV-Indiana wild type; lane 2, tsG22; lane 3, tsG31; lane 4, tsG41; lane 5, VSV-New Jersey (Missouri); lane 6, VSV-New Jersey (Ogden). Arrows indicate the positions of the viral proteins.*

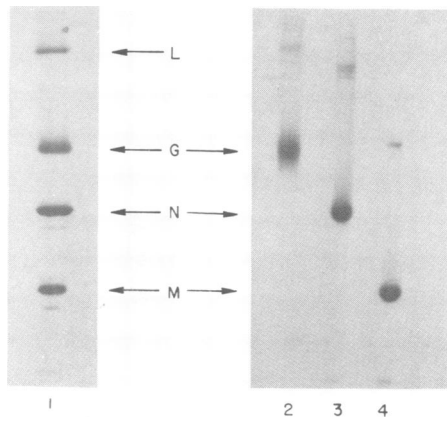

FIG. 2. *Qualitative analysis of VSV proteins isolated from preparative gels. VSV proteins were separated on preparative gels (Fig. 1) and the individual species were excised. Aliquots were re-electrophoresed separately on a 10% discontinuous polyacrylamide slab gel at 20 mA for 5.5 h. The gel was dried and radioautographed for 24 h with Kodak XR-5 medical X-ray film. Lane 1, VSV marker proteins; lane 2, G protein; lane 3, N protein; lane 4, M protein.*

tants with wild-type virion proteins. Earlier experiments indicated that the peptide pattern obtained by digestion with V8 protease is sensitive to the time of enzymatic digestion and the enzyme-to-substrate ratio (2). In our experiments, the time of enzymatic digestion was not varied, and proteolysis was dependent only on the time of enzyme-substrate comigration through the stacking gel. Under the standard conditions of electrophoresis, this was equal for all samples, as could be ascertained from the rate of movement of the marker dye in the stacking gel. To establish the best conditions for detecting differences between mutant and wild-type proteins, enzyme-to-substrate levels were varied over a range of 1:5 to 1:500. As expected, with increasing enzyme-to-substrate ratios, the large peptide bands disappeared and were replaced by intermediate- and smaller-sized peptide bands. Depending on whether the detectable differences between the wild-type and mutant proteins were among the small or large peptides, the appropriate high or low enzyme-to-substrate ratio was selected to enhance these differences (Fig. 3C and D). In Fig. 3C, peptide profiles of wild-type virion (lane 1) and *ts*G31 mutant (lane 2) M proteins, digested at an enzyme-to-substrate ratio of 1:500, are shown. Under these conditions, no difference in the profiles is discernible. When the enzyme-to-substrate ratio was increased to 1:50, a new band appeared among the low-molecular-weight peptides of the mutant protein (Fig. 3C, lane 4, arrow), which was absent in the corresponding wild-type profile (Fig. 3C, lane 3, arrow). A greater increase in enzyme concentration (1:5) enhanced the differences, as shown in Fig. 3D. The profiles in this figure also demonstrated that the two peptides, absent in the wild-type (lane 1), were detectable in two members of group III mutants, *ts*G31 (lane 2) and *ts*G33 (lane 3). A revertant derived from mutant *ts*G31 exhibited the wild-type protein profile (lane 4), suggesting that the reversion resulted in a change of the mutated amino acid to one with characteristics similar to the wild-type amino acid. Under similar conditions, no differences in the N and G proteins of wild-type virion and a group III mutant were detectable (Fig. 3A and B).

Partial protease digestion of wild-type virion, group IV mutant, and revertant N proteins. Peptides of the N protein of *ts*G41, a group IV mutant, were compared with those obtained by partial digestion of the corresponding wild-type virion and revertant protein. A peptide of intermediate size which was present in the mutant (Fig. 4, lane 3, arrow) was absent in the wild-type virion (Fig. 4, lane 1) and in a revertant derived from *ts*G41 (Fig. 4, lane 2).

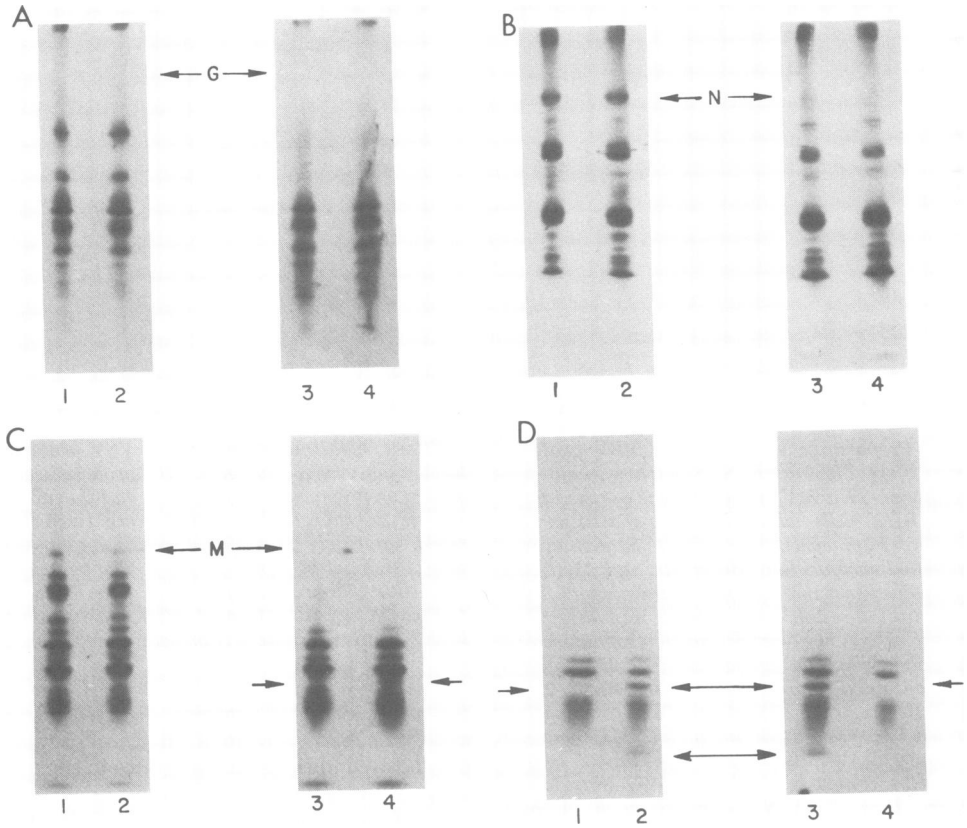

FIG. 3. *Limited digestion of G, N, and M proteins from VSV wild type, tsG31, tsG33, and a revertant of tsG31. Protein bands excised from 8% polyacrylamide gels (Fig. 1) were equilibrated in 10 ml of 0.125 M Tris-hydrochloride (pH 6.8)-0.1% SDS-1 mM EDTA for 30 min. The individual bands were bisected, and each half was placed in a well of a discontinuous slab gel containing a 3.5-cm stacking gel on top of an 8-cm, 15% polyacrylamide running gel. The sample was overlaid with 20 μl of equilibration buffer containing 20% glycerol. Various amounts of S. aureus protease V8 were added in equilibration buffer containing 10% glycerol. Electrophoresis was at 20 mA for 6 h. The gel was then fixed for 15 min in 10% acetic acid-10% glycerol, dried, and autoradiographed for 6 days with Kodak XR-5 medical X-ray film. (A) G protein of wild-type VSV (lane 1) and tsG31 (lane 2) at an enzyme-to-substrate ratio of 1:500. G protein of wild type (lane 3) and tsG31 (lane 4) at an enzyme-to-substrate ratio of 1:50. (B) N protein of wild-type VSV (lane 1) and of tsG31 (lane 2) at an enzyme-to-substrate ratio of 1:500. N protein of wild type (lane 3) and of tsG31 (lane 4) at an enzyme-to-substrate ratio of 1:50. (C) M protein of wild-type VSV (lane 1) and of tsG31 (lane 2) at an enzyme-to-substrate ratio of 1:500. M protein of the wild type (lane 3) and of tsG31 (lane 4) at an enzyme-to-substrate ratio of 1:50. (D) M proteins of wild-type VSV (lane 1), tsG31 (lane 2), tsG33 (lane 3), and a revertant of tsG33 (lane 4) at an enzyme-to-substrate ratio of 1:5. The letter-marked arrows in A, B, and C indicate the positions of the respective undigested proteins. The unmarked arrows in C and D indicate differences in peptide bands.*

This peptide rapidly disappeared when the enzyme-to-substrate ratio was increased from 1:500 to 1:50 (Fig. 4, lanes 4 to 6). No differences were found upon examination of the G and M proteins (data not shown).

Analysis of a group II mutant. Although [^{35}S]methionine of the highest available specific activity was utilized, sufficient label was not incorporated into the NS protein for a partial peptide hydrolysate analysis. However, high yields of radioactive NS protein were obtained through the exploitation of its phosphoprotein nature by use of $^{32}P_i$. It should be noted, however, that the analysis was limited to the peptide regions adjacent to the phosphate groups, which may not be as numerous as the methionine residues in this and other virion proteins.

Profiles of partial protein hydrolysates of the NS component of wild-type virion, group II mutant (*ts*G22), and a revertant isolated from the latter are shown in Fig. 5. At low enzyme concentration, a low-molecular-weight peptide (ar-

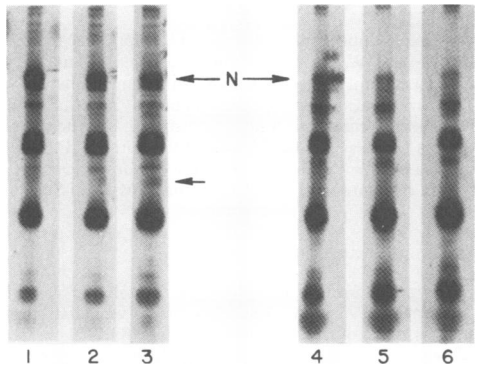

FIG. 4. *Limited digestion of the N protein of wild-type VSV, a group IV ts mutant, and its revertant. The ^{35}S-labeled N proteins were isolated from 8% polyacrylamide slab gels as described in Fig. 1. Digestion, electrophoresis, and autoradiography were as described in the legend to Fig. 3. Lanes 1, 2, and 3: N proteins of wild-type VSV, the tsG41 revertant, and tsG41 mutant, respectively, digested at an enzyme-to-substrate ratio of 1:500. Lanes 4, 5 and 6: N proteins of wild-type VSV, tsG41 revertant, and tsG41 mutant, respectively, at an enzyme-to-substrate ratio of 1:50. The letter-marked arrow indicates the position of undigested N protein, and the unmarked arrow indicates a difference observed in the peptide profiles.*

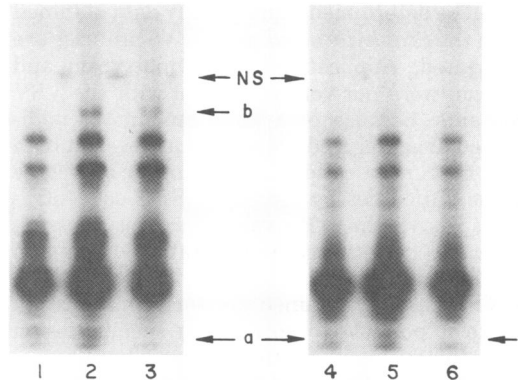

FIG. 5. *Limited digestion of the NS proteins derived from wild-type VSV, a group II ts mutant, and its revertant. Virus-infected BHK cells were incubated in phosphate-free media made up to 50 μCi of $^{32}P_i$ per ml. The ^{32}P-labeled NS protein was isolated by 8% polyacrylamide gel electrophoresis, similar to that shown in Fig. 1. The slower-moving major phosphorylated band was excised and used in the limited proteolysis, which was performed as described in the legend to Fig. 3. Lanes 1, 2, and 3: NS protein of wild-type VSV, tsG22 revertant, and tsG22 mutant, respectively, at an enzyme-to-substrate ratio of 1:5,000. Lanes 4, 5, and 6: NS protein of wild-type VSV, tsG22 revertant, and tsG22 mutant, respectively, at an enzyme-to-substrate ratio of 1:500. Arrows marked a and b indicate differences in the peptide patterns, and the arrow marked NS locates the position of the undigested protein.*

row a) was present in the wild-type and revertant protein hydrolysates (lanes 1 and 2) but was absent in the mutant peptide profile (lane 3). At higher enzyme-to-substrate ratios (1:500), this difference was slightly more enhanced (lanes 4, 5, and 6; wild-type, revertant, and mutant virion, respectively). In addition to this difference, at low enzyme concentration the mutant profiles contained a band corresponding to a high-molecular-weight peptide which was absent from the wild-type virion profile (arrow b). This peptide seemed to be present in the revertant profile (lane 2) and might therefore be due to a mutation irrelevant to the phenotype.

DISCUSSION

Because of pleiotropic effects often encountered in mutants of animal viruses, the site of mutation is frequently difficult to determine. Complex and time-consuming experiments are sometimes required to obtain evidence implicating a protein as being mutated. Some experiments of this nature applied in the characterization of VSV *ts* mutants were mentioned in the introduction. The fastest and most informative method appears to be the study of the in vivo stability of viral proteins which is often decreased as a result of the mutation (13). However, this method does not seem to be generally applicable, as evidenced by its failure to assign a defective protein to VSV *ts* mutants of group II (13). Similarly, complete proteolytic digestion and analysis of the ensuing peptides are in most cases too insensitive to demonstrate a single amino acid replacement, unless the latter is involved in the specificity of the enzymatic digestion. However, a method sensitive to conformational changes of proteins is likely to be useful since the biological activity of the mutant protein is probably impaired by such a change. During incomplete enzymatic hydrolysis, the degree of accessibility of a specific site is very much affected by the general conformation of the protein. This approach was successfully used in this paper to demonstrate differences between wild-type and *ts* mutants of VSV. The enhancement of these differences very much depends on the enzyme-to-substrate ratio as demonstrated by all the data, but especially by those presented in Fig. 3. The selection of the correct enzyme-to-substrate ratio appeared to be the most critical part of these analyses. Since a large number of samples could be electrophoresed on a single gel, the advantageous enzyme concentration range

could be determined rapidly. Only data obtained with the *Staphylococcus aureus* V8 protease are presented. Applications of chymotrypsin and trypsin were not very successful with the VSV mutants, because of a large amount of undigested or aggregated proteins found even at high enzyme concentrations. An attempt to overcome these difficulties by increasing SDS concentrations above the 0.1% value was unsuccessful, probably because of the generation of random protein conformations.

The results obtained in this investigation make it possible to correlate a specific lesion with the complementation groups whose representative mutants were examined. For the first time a positive identification of a defective NS protein for group II has been made. The assignment of M and N protein lesions to groups III and IV, respectively, are in agreement with less direct methods reported in the literature (13, 15, 16, 21). Although some of the *ts* mutants examined in this study have undergone repeated laboratory passages, there was no indication of silent mutations which would interfere with the conformational analyses of the proteins. This observation is also consistent with comparative oligonucleotide mapping, which suggested that most differences in VSV isolates were due to differences in biological origin and not to a large number of passages in the laboratory (4). Nevertheless, the NS peptide profile of *ts*G22 and its revertant suggested that a silent mutation may have taken place in this cistron.

The reversion of the mutants, which resulted in peptide profiles identical with the wild type, may be interpreted in one of two ways. Either the back mutation restored the wild-type amino acid residue or a second mutation (suppressor mutation) restored the original conformation of the molecule. If the peptide pattern were only sensitive to conformation and not to primary structure of these revertants, the results would not distinguish between these two possibilities.

Our *ts* mutants of VSV were originally derived from the wild-type virus by chemical mutagens and, as such, are presumably characterized by a single amino acid replacement. The applicability of Cleveland's method to the detection of one-step mutations suggests that this method may be of general usefulness in the analyses of viral mutants. We have also applied this method for characterizing the two recently described VSV subgroups of the New Jersey serotype, which exhibit considerable differences in their protein structure (1, 20, 24). Significant variations between the profiles of partial hydrolysates of several proteins from these two subgroups were observed (unpublished data).

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