Effect of Trypsin and Chymotrypsin on the Polypeptides of Large and Small Plaque Variants of Foot-and-Mouth Disease Virus: Relationship to Specific Antigenicity and Infectivity

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SUMMARY

Large and small plaque variants of A12 foot-and-mouth disease virus were shown to have specific antigenic determinants. Large plaque virus antigenic specificity was destroyed by trypsin treatment, but the small plaque antigen was resistant despite cleavage of the trypsin-sensitive polypeptide. The cleavage of polypeptide VP₃ by trypsin resulted in the formation of a new antigen not present on untreated virus. The effects of chymotrypsin and trypsin on the polypeptides of the plaque variants have been examined and related to changes in antigenicity, infectivity, and exposure of the polypeptides at the surface of the capsid. The results are discussed in relation to the orientation of the trypsin-sensitive polypeptide in the virus capsid.

INTRODUCTION

The large plaque (LP) and small plaque (SP) variants of several serotypes of foot-and-mouth disease virus (FMDV) have different antigenic characteristics (Cowan, 1969; McVicar & Sutmoller, 1972a; Cowan et al. 1974). Virus passed on monolayer or suspension cultures of baby hamster kidney (BHK)-21 cells selectively produced LP and SP variants, respectively (Cowan et al. 1974). In similar experiments, Meloen (1976) reported that O₁ FMDV grown in BHK suspension cultures and virus produced in the Frenkel culture system of bovine tongue epithelium differed antigenically. Trypsin-treated virus from Frenkel culture (Frenkel, 1950) lost its specific antigenicity and appeared identical on immunodiffusion to the suspension culture virus. This finding, coupled with information that trypsin removes a site on the virion associated with immunogenicity (Brown & Smale, 1970), led to the conclusion that the suspension cell virus was antigenically deficient and had lost the trypsin-sensitive site. However, Cowan et al. (1978) have shown that both LP and SP viruses have unique antigenic properties associated with the trypsin-sensitive site on the virion. This indicated that SP virus was not deficient antigenically but was different from LP virus.

Results in the present report confirm the distinct antigenic properties of LP and SP variants using A₁₂ FMDV. The presence of the trypsin-sensitive virus polypeptide is shown for each variant. In addition, differences in polypeptide cleavage with enzyme treatment are shown and related to changes in antigenicity and infectivity.

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METHODS

Reagents. Trypsin treated with the chymotrypsin inhibitor L-(tosylamide 2-phenyl) ethyl chloromethyl ketone (TPCK), \( \alpha \)-chymotrypsin and soybean trypsin inhibitor were obtained from Worthington Biochemical Corp., Freehold, New Jersey. Trypsin, 1:300 was supplied by Nutritional Biochemicals Corp., Cleveland, Ohio, and \( p \)-tosyl-L-arginine methyl ester (TAME) from Sigma Chemical Corp., St Louis, Missouri. \(^{125}\)Iodine (as sodium iodide in pH 8 to 11 dilute NaOH) was obtained from Amersham-Searle, Arlington Heights, Illinois.

Viruses. The LP (ab-antigenic type) and a SP variant of \( A_{12} \) strain 119 FMDV were used in this study (Cowan, 1969). The variants were additionally plaque isolated three times on primary calf kidney culture (Bachrach et al. 1962) for inoculum stocks. Some tests were confirmed with the LP b-antigenic type (Cowan, 1969) and with LP and SP isolates obtained from \( A_{12} \) FMDV which had been in continuous cell passage (Bachrach et al. 1975). The plaque-size characteristics of the isolates were confirmed with overlays of agar and agar containing 1 mg/ml of DEAE-dextran (Martinsen, 1970), and the antigenic type was confirmed by immunodiffusion analysis (Cowan, 1969).

Virus growth and purification. Virus was produced by two passages on BHK-21 cell monolayers (Polatnick & Bachrach, 1964) in Hanks' lactalbumin hydrolysate (HLH), concentrated by precipitation with polyethylene glycol 6000 (PEG; Cowan et al. 1974) and resuspended in barbital-glycine buffer (Cowan & Graves, 1966) containing an additional 0.15 M-NaCl necessary to maintain solubility of the SP variant (Cowan et al. 1974). The PEG-concentrated viruses were purified by ultracentrifugation into CsCl gradients (Wagner et al. 1970) and dialysed against tris-buffered NaCl solution (TBS, 0.05 M-tris-HCl, pH 7.5, 0.3 M-NaCl). Virus concentration was determined spectrophotometrically (Bachrach et al. 1964).

Virus uniformly labelled with a mixture of \(^{14}\)C-amino acids was grown by inoculating cultures at high multiplicity of infection (10:1) and addition of label as previously described (Moore, 1977). Purification was as for unlabelled virus above.

Enzyme treatment. Purified viruses were incubated at 37 °C with freshly prepared enzyme solutions in TBS at dosages and times indicated in the Results. Treated samples were re-purified on sucrose gradients free of enzyme (Bachrach et al. 1975) for immunodiffusion and polypeptide analysis.

Enzyme effects on infectivity were tested with virus stocks from calf kidney culture diluted 1:100 in TBS. The purpose of this procedure was to dilute extraneous protein and to avoid possible aggregation-disaggregation effects with concentrated purified virus preparations. At appropriate times after addition of enzyme and incubation, test samples were diluted 10-fold into ice-cold HLH containing soybean trypsin inhibitor. Enzyme-free control samples were treated similarly. Infectivity changes were measured by plaque assay on secondary calf kidney cultures in 35 mm culture dishes overlaid with agar-DEAE-dextran (Martinsen, 1970). Soybean trypsin inhibitor did not significantly affect virus infectivity.

Equivalent enzyme activity doses of TPCK-treated and untreated trypsin were determined by spectrophotometric determination of enzyme activity with the substrate TAME (Hummel, 1959). Activity of TPCK-trypsin was 245 units/mg and untreated trypsin, 10 units/mg. Alpha-chymotrypsin activity was 61 units/mg, as specified by the manufacturer.

Antisera. Antisera to LP and SP variants were collected 7 days post-infection (p.i.) after intradermal footpad injection in guinea pigs (Cowan, 1969). Also, 7-day sera were produced by subcutaneous inoculation of 25 \( \mu \)g of purified virus isolate emulsified in incomplete
Freund's adjuvant (Cowan, 1968). The same variant recognition capability shown in Results was obtained with antisera prepared by both methods. Guinea pig hyperimmune virus antiserum was produced as previously described (Cowan, 1969). Chymotrypsin- and trypsin-treated viruses in complete Freund's adjuvant were inoculated subcutaneously in 25 µg doses and antisera collected at 30 days post-inoculation (Cowan, 1969). Antisera to the enzyme-treated viruses were absorbed at slight antigen excess with untreated virus to remove virus-reactive antibody. The approximate equivalence point was determined by immunodiffusion analysis of supernatant fluids in a test absorption series for unreacted virus or antibody (Sutmoller & Cowan, 1974).

**Immunodiffusion analysis.** Ouchterlony type immunodiffusion tests were performed in 1% agar (Cowan & Graves, 1966), with an additional 0.15 M- NaCl added to the agar (Cowan et al. 1974).

**Polyacrylamide gel electrophoresis.** The polypeptides of the A12 FMDV sample were resolved on 12.5% polyacrylamide gels containing 0.1% sodium dodecyl sulphate (SDS) and 8 M-urea (Bachrach et al. 1975); 9 cm long, 1.27 mm thick slab gels were used rather than cylindrical gels (except Fig. 4b). The gels were fixed in 50% trichloroacetic acid for 1 h and washed in 25% propanol and 10% acetic acid before they were stained with Coomassie brilliant blue. This method caused the trypsin-sensitive polypeptide to migrate as VP3 rather than as VP2 as in an earlier method (Vande Woude et al. 1972) or elsewhere as VP1 (Wild et al. 1969). To avoid confusion, in this report the trypsin-sensitive polypeptide is referred to as VP3.

Mol. wt. of virus polypeptide VP3 and fragments of VP3 produced by enzyme cleavage were determined by comparison with marker proteins (bovine serum albumin, IgG heavy chain, aldolase, chymotrypsinogen, pancreatic ribonuclease, cytochrome c) on gel slabs as above, with the exception that they contained 15% acrylamide and only 0.5 M-urea. The mol. wt. values are presented in the Results in abbreviated form (e.g. p14.9 for mol. wt. 14900). Mol. wt. values assigned to the peptide fragments are relative considering the method used. However, the differences were certain and reproducible from the side-by-side analyses on slab gels. Thus, the values are given in the Results to illustrate these differences.

The relative recovery of the weight of VP3 in enzyme-cleaved products of VP3 was also determined by counting the radioactivity recovered in the various peptides of 14C-amino acid labelled FMDV. Cylindrical 0.6 cm diam. gels containing 11% polyacrylamide, 0.1% SDS and 8 M-urea were electrophoresed, divided into 1 mm fractions and radioactivity was determined by liquid scintillation counting.

**Labelling of virus particles with 125I.** The general method of Hunter & Greenwood (1962) was used to label 50 µg of virus with 100 µCi of Na125I by addition of 10 µg of chloramine-T. After 1 min, the reaction was stopped with sodium metabisulphite, and excess KI and bovine serum albumin were added. Before the 125I-labelled samples were electrophoresed on slab gels, intact labelled virus was re-purified (as with trypsin-treated purified virus above) to eliminate soluble 125I and virus material possibly degraded during labelling. Labelled virus samples were run on SDS-polyacrylamide gels containing 8 M-urea and 10% acrylamide, the gels were then fixed and washed, dried onto filter paper at 80 °C under vacuum and applied to X-ray film to locate the labelled virus polypeptides.
RESULTS

Effect of enzyme treatment on virus antigenicity

The LP and SP isolates of A₁₂ FMDV were compared by immunodiffusion with 7-day p.i. sera prepared to each of the viruses. Each antiserum precipitated the two isolates, but a specific spur line that formed over the heterologous isolate showed that each carried a characteristic LP or SP antigenic determinant (Fig. 1a). When trypsin-treated isolates were compared (LP antiserum), the trypsined large plaque (T-LP) virus had lost the specific antigenicity, and the precipitin line coalesced with the trypsined small plaque (T-SP) virus (Fig. 1b). However, the SP-specific antigenicity (SP antiserum) remained despite trypsin treatment, even with a higher concentration of TPCK-trypsin (245 units/ml) or with 10 units/ml of trypsin not treated with the chymotrypsin inhibitor TPCK (Fig. 1c). The trypsin resistance of SP-specific antigenicity was also apparent when T-SP was com-
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Fig. 2. Change in antigenicity of LP (a) and SP (b) FMDV with enzyme treatment. TPCK-trypsin and chymotrypsin as in Fig. 1; HI CHY- indicates treatment with 10 units/ml chymotrypsin for 1 h at 37 °C. Antisera were 7-day p.i. aLP (a) and aSP (b).

pared to untreated LP virus (not shown). This finding indicated a true resistance to loss of the specific antigen excluding the possibility of an artefact caused by unequal loss of a shared trypsin-sensitive antigen or the appearance of a new T-SP specificity not found on T-LP virus. The above results are similar to the individual antigenic specificity of LP and SP viruses reported for Asia-1 and O1 FMDV, but the trypsin resistance of the A12 SP antigen found here differs from the reported uniform susceptibility of LP and SP viruses to trypsin treatment (Cowan et al. 1978).

The difference in sensitivity of the specific antigens of LP and SP viruses to trypsin was confirmed with the LP b-antigenic variant of A12 FMDV and additional recently isolated LP and SP stocks from A12 FMDV (see Methods). In each experiment the LP-specific antigen was lost and the SP antigen remained after trypsin treatment (not shown).

Treatment of both LP and SP isolates with chymotrypsin had no detectable effect on their specific antigenicity (Fig. 1 d) and extended treatment (10 units/ml, 1 h) did not reduce the degree of specific spurring of either isolate.

The difference in sensitivity of the specific antigens of LP and SP viruses to enzyme treatment was supported by a comparison of LP and SP virus with each of their respective trypsin and chymotrypsin products (Fig. 2). Consistent with the loss of specific antigenicity with trypsin, LP virus produced a strong spur-line over T-LP virus indicating a considerable loss or change in antigenicity (Fig. 2a). Chymotrypsin treatment altered the antigenicity less with only a slight spur showing from the LP precipitin line. More extensively treated LP virus allowed a larger spur to form, but both chymotrypsin-treated LP preparations maintained a strong spur line over T-LP virus comparable to the LP-T-LP spur. Trypsin and chymotrypsin treatment of SP virus had little effect on its antigenic reactivity (Fig. 2b). A precipitin line of identity formed with untreated and treated SP virus; only a faint spur-line showed from the SP virus over the treated samples. Thus, the stability of the SP-specific antigen after enzyme treatment was reflected by a lack of loss (or change) in antigenicity shown here.

The loss of LP antigenicity shown above supports previous findings that trypsin removes an antigenic site on the virus particle (Wild et al. 1969). Multiple antigenic sites were shown
Fig. 3. Demonstration of the trypsin-specific antigen on LP and SP FMDV treated with trypsin, but not with chymotrypsin: (a) a trypsin-specific antigen shown only with trypsin-virus antiserum (aT-V) and untreated virus absorbed trypsin virus antiserum (VIR ABS aT-V), but not hyper-immune serum (HYP) and 7-day p.i. serum; (b) LP trypsin-specific antigen; (c) SP trypsin-specific antigen; (d) chymotrypsin virus antiserum (aCHY) showing no specific anti-trypsin or anti-chymotrypsin virus activity on absorption with untreated virus (VIR ABS aCHY). Enzyme-treated viruses labelled as in Fig. 1.

on the virus particle, including one that was present after trypsin treatment and distinct from the antigen removed by trypsin (Brown & Smale, 1970; Rowlands et al. 1971). However, these studies did not show whether the antigen was newly formed and specific for trypsinized virus or shared on untreated virus particles. To test this, antiserum prepared with trypsinized $\Lambda_{12}$ FMDV was compared with hyperimmune and 7-day p.i. LP antiserum by immunodiffusion with LP and T-LP virus (Fig. 3a). Hyperimmune serum did not distinguish between the two viruses and the 7-day p.i. serum showed a loss of antigenicity seen above with T-LP virus (Fig. 2a). Antiserum to trypsinized virus reacted like hyperimmune serum with the exception that a spur-line formed from T-LP over the LP virus precipitin line. When trypsin-virus antiserum was absorbed with untreated virus, reactivity remained
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Effect of enzyme treatment on the polypeptides of LP and SP virus

Treatment of LP virus with 10 units/ml of trypsin or TPCK-trypsin completely cleaved VP₃ (approx. p25) to smaller fragments that were retained in the virus particle (Fig. 4a). The sizes of the fragments were different with the two enzyme preparations: p14.9 for TPCK-trypsin and p14.5 (trace p16.8) for untreated trypsin. TPCK-trypsin cleavage of VP₃ was complete, and the size of the fragment the same whether at a previously used lower dose of about 1 unit/ml (Bachrach et al. 1975) or of 245 units/ml (not shown). The cleavage product was seen as a single band and not as a closely spaced pair as previously reported (Bachrach et al. 1975). However, in most tests, the staining intensity of the cleaved VP₃ product indicated that the original amount of VP₃ protein was present (Fig. 4b). Tests for ¹⁴C-activity in trypsin cleaved VP₃ indicated that 77% of the original VP₃ was recovered as p14.9. Trypsinization of ¹⁴C-virus also produced a quantity of radioactivity smaller than VP₄ which, if all derived from VP₃, represented 20% of VP₃.

which was specific for trypsin-treated virus. Thus, although trypsin-treated virus induced antibody reactive with intact virus, it also induced antibody activity specific for an antigen on trypsinized virus alone.

Enzyme-treated LP and SP viruses were each examined for the appearance of the trypsin-specific antigen. Both T-LP and T-SP viruses produced a trypsin-specific spur line over untreated virus, and trypsin virus-specific antiserum (intact virus absorbed) precipitated only T-LP and T-SP virus (Fig. 3b, c). The lack of reaction with chymotrypsin-treated LP or SP virus indicated that the trypsin-specific antigen was not induced by this enzyme. Also, chymotrypsin-treated virus did not acquire a ‘chymotrypsin-specific’ virus antigen. Antiserum prepared to chymotrypsin-treated virus showed a coalescing precipitin line with LP or SP virus and their respective enzyme-treated products (for example, LP virus, Fig. 3d). When absorbed with untreated virus, the antiserum lost reactivity with all LP virus preparations. The experiments above indicated that the persistence of SP-specific antigenicity was not due to a lack of trypsin effect on the capsid, for both LP and SP virus showed the appearance of the trypsin-specific antigen.

Fig. 4. SDS-polyacrylamide gel electrophoresis of LP FMDV polypeptides after enzyme treatment: (a) 15 min at 37 °C with 5 units/ml chymotrypsin (CHY), 10 units/ml TPCK-trypsin (T), 10 units/ml trypsin (crT), and untreated virus (LP); (b) cylindrical gels showing recovery of cleaved polypeptides, trypsin-treated as in (a); (c) additional cleavage with a 1 h chymotrypsin treatment at 10 units/ml (HI CHY). On the left, (VP₃) indicates enzyme-cleaved VP₃ products.
Treatment of LP virus with chymotrypsin (5 units/ml, 15 min) produced a fragment, larger than the trypsin products, of p15-4 and a smaller amount of p14-5, equivalent to the trypsin (not TPCK-treated) product (Fig. 4a, c). Some VP3 remained uncleaved. More extensive chymotrypsin treatment (10 units/ml, 1 h) cleaved VP3 completely and produced mostly the smaller fragment p14-5 and some p15-4 (Fig. 4c).

Enzyme treatments of the SP isolate produced an array of mol. wt. fragments of VP3 similar to that seen with LP virus (Fig. 5a). However, before enzyme treatments, most samples of purified SP virus showed partial cleavage of VP3 to a p15-0 fragment. Virus produced in BHK-21 cells from another source or in primary calf kidney cultures contained the fragment. The VP3 appeared to cleave during incubation in the culture fluid, as early harvests or virus collected from the cell cytoplasm yielded virus with most VP3 intact and little cleavage product. On standing at 4 °C, purified samples of SP virus lost additional VP3 to the p15-0 fragment. The LP isolate appeared to be more stable; little cleavage product ever appeared on storage at 4 °C.

Treatment of the SP isolate with chymotrypsin increased the amount of p15-0 fragment already present in untreated SP virus. Also, some intact VP3 remained (Fig. 5a, b) and traces of p14-3 and p19-0 fragments were formed. As with LP virus, more extensive chymotrypsin treatment (10 units/ml, 1 h) cleaved the balance of VP3 (Fig. 5b). Unlike LP virus which then contained principally the smallest species, about equal parts of the three SP cleavage products were formed.

Trypsin treatment of SP virus caused complete cleavage of VP3 to a fragment p14-6 with TPCK-trypsin and to p14-3 (plus trace p17-4) with trypsin (Fig. 5a). Treatment of SP virus in which most of the VP3 had spontaneously degraded to p15-0 yielded either p14-6 or p14-3 according to the type of trypsin used. Insufficient intact VP3 was originally present to account for the amount of trypsin-cleaved products found. Therefore, the p15-0 fragment found in untreated virus must have been retained in the capsid and cleaved further, along with the intact VP3, to form the smaller products. The smaller VP3 fragment produced by trypsin versus TPCK-trypsin appeared to result from additional contaminating chymotrypsin activity. A mixture of 10 units/ml TPCK-trypsin plus 5 units/ml of chymotrypsin produced the p14-3 and p17-4 fragments typical of untreated trypsin except that more of the larger species was generated (Fig. 5c).
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Fig. 6. Loss of infectivity with enzyme treatment of LP (a) and SP (b) FMDV. Test samples contained 10 units/ml of TPCK-trypsin (T), trypsin (crT), chymotrypsin (CHY), or control incubated only in buffer (CK). One sample contained 1 units/ml TPCK-trypsin [T(1 unit)].

The enzyme treatments described above produced different cleavage products of VP₃. However, the enzyme effects were specific for VP₃. Polypeptides VP₁, VP₂ and VP₆, VP₄ (both not apparent in the figures) were not altered in mobility by any of the enzyme treatments.

**Infectivity of enzyme-treated LP and SP virus**

Infectivity of LP and SP virus was significantly reduced by trypsin treatment. When LP virus was treated with 10 units/ml of trypsin or TPCK-trypsin, infectivity dramatically decreased in the first 5 min of incubation and changed little after that (Fig. 6a). The SP virus showed a similar loss of infectivity complete by 5 min, but less infectivity was lost with TPCK-trypsin (Fig. 6b). This difference between LP and SP virus was more apparent when 1 unit/ml of TPCK-trypsin was used. As shown by polyacrylamide gel electrophoresis, this dose of TPCK-trypsin was sufficient to completely cleave VP₃ in preparations of purified virus containing much larger amounts of virus protein (Bachrach et al. 1975).

Chymotrypsin had little effect on infectivity of either LP or SP virus in the first 15 min of incubation (Fig. 6). However, by 1 h, LP virus infectivity loss was extensive. The time of the infectivity loss coincided with the conversion of the p154 fragment and residual VP₃ to
the smaller p14.5 fragment (Fig. 4c). The SP virus infectivity was less affected through the 1 h incubation period. This difference probably reflects the limited conversion of the p15.0 fragment to the smaller p14.3 fragment seen with prolonged chymotrypsin treatment (Fig. 5b). The maintenance of infectivity in SP virus particles containing VP3 as p15.0 was supported by the finding that some untreated SP virus samples contained this fragment and little intact VP3. However, these preparations had high infectivity titres comparable to those of other preparations containing virus with intact VP3.

**Labelling of polypeptides in enzyme-treated viruses**

Treatment of the LP and SP isolates of A12 FMDV with trypsin, chymotrypsin or a mixture of the two cleaved only VP3. Also, when intact FMD viruses were labelled in vitro with 125I, the trypsin-sensitive peptide (here VP3) accumulated most of the label (LaPorte & Lenoir, 1973; Bachrach et al. 1975). To determine whether VPs cleavage caused the other polypeptides to become exposed at the surface of the capsid, enzyme-treated virus particles were tested for incorporation of 125I into tyrosine of the virus polypeptides (Fig. 7). Although all of the polypeptides became labelled when solubilized in 8 M-urea, VP3 was principally labelled in situ in intact particles. With enzyme-treated particles, the cleaved VP3 fragments became labelled. In untreated viruses, polypeptide VP2 showed a small amount and VP1 a trace (not visible in Fig. 7) uptake of 125I. Uptake of 125I did not increase in VP1 and VP2 with enzyme-treated particles. This finding indicated that tyrosine residues of these polypeptides did not become surface orientated.

**DISCUSSION**

The results presented here with A12 FMDV support previous findings for Asia-1 and O1 FMDV that, in addition to common antigenic determinants, LP and SP isolates of FMDV show specific antigenic differences (McVicar & Sutmoller, 1972a; Cowan et al. 1974, 1978). The concept of an antigenic deficiency in the trypsin-sensitive polypeptide introduced by
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Meloen (1976) seems inaccurate. The virus grown in BHK suspension culture was thought to have ‘lost’ the trypsin-sensitive antigen. An immunodiffusion reaction of identity was found for suspension culture virus and trypsin-treated Frenkel culture virus, the Frenkel virus known to have lost an immunogenic site (Brown & Smale, 1970). However, when antisera to LP and SP variants were used to compare the variants, each was shown to contain antigenically different trypsin-sensitive determinants (Cowan et al. 1978). The results presented here indicate that LP and SP variants of A12 FMDV contain the trypsin-sensitive polypeptide (here VP₃) and also that the SP variant has its own specific antigenicity, different from that of LP virus.

The resistance of SP virus-specific antigenicity and smaller loss of infectivity with trypsin treatment differ from most reported results. Generally, effects of trypsin on FMDV were consistent and showed cleavage in one polypeptide and an associated loss of infectivity, antigenicity, immunogenicity and ability to absorb to cell receptors (Wild & Brown, 1967; Strobbe et al. 1974; Cavanagh et al. 1977). Specific LP and SP antigens of Asia-1 and O₁ FMDV were lost with trypsin treatment (Cowan et al. 1978) and for O₁ FMDV (Frenkel) specificity was similarly lost (Meloen, 1976). In the present tests, specific antigenicity of SP virus was not destroyed, despite the complete cleavage of VP₃ by trypsin. Additional examples of resistance of FMDV to trypsin have been reported. Trypsinized A₁₂ (Bachrach et al. 1975) and C₃ (Rowlands et al. 1971) FMDV produced about as much virus-neutralizing antibody in guinea pigs as untreated virus. Purified C₃ FMDV infectivity was also resistant to trypsin despite polypeptide cleavage (Rowlands et al. 1971; H. L. Bachrach, personal communication). These results indicate that other FMDV serotypes and strains may vary in their susceptibility to trypsin treatment.

In contrast to the trypsin effect, the specific SP and LP antigenicities were stable to chymotrypsin treatment. Also, no specific chymotrypsin-induced antigen was found comparable to the trypsin-specific antigen. With prolonged treatment, chymotrypsin caused further VP₃ cleavage to a smaller mol. wt. fragment. The conversion to the smaller polypeptide was more complete with LP virus and was reflected in a greater infectivity loss. These results agree with those of Cavanagh et al. (1977) in which a shift from mol. wt. 20000 to 18000 during chymotrypsin treatment was associated with loss of infectivity, cell absorption and, to a lesser degree, immunogenicity.

The principal enzyme cleavage products of A₁₂ FMDV (mol. wt. 14000 to 15000) found in the present study (also Bachrach et al. 1975) differ from the fragments with mol. wt. 18000 to 20000 plus <10000 found for O₁ FMDV (Strobbe et al. 1974; Cavanagh et al. 1977). The difference probably reflects locations of susceptible peptide bonds exposed on the capsid surface of the two serotypes. The present results do not establish whether the single band seen with trypsin cleavage of VP₃ represented two polypeptides migrating together or a single species. The 25000 mol. wt. assigned VP₃ in the present study is lower than mol. wt. 27000 to 30000 determined by SDS-gel electrophoresis (Vande Woude et al. 1972) and C-terminal analysis (Bachrach et al. 1973). Two co-migrating fragments of mol. wt. 15000 would appear to exceed the size of the parent VP₃ polypeptide. However, a recovery of 77% of the original ¹⁴C-labelled VP₃ would set a single trypsin fragment at approx. 19000 to 23000, a size not supported by comparison with standard protein markers. The difference may arise from a limited elution and degradation of VP₃ fragments from an equal-sized pair in the virus capsid.

The presence of the mol. wt. 15000 polypeptide fragment in untreated SP virus did not affect its infectivity or antigenicity. In fact, large amounts of this fragment that formed spontaneously or with chymotrypsin treatment caused no significant changes in properties.
The tendency to form cleavage products does not mean the SP virus was deficient in VP₃, but that it may have contained a principal chymotrypsin-specific site more exposed than on LP virus.

In this report, VP₃ was cleaved by chymotrypsin, trypsin or a mixture to principally three different size classes of polypeptide fragments. Changes or lack of changes in infectivity and antigenicity were associated with the different products. The difference in mol. wt. of the largest and smallest fragments was less than mol. wt. 1000 for both variants. Although this difference in size could represent an antigenic determinant, physical loss of an antigenic segment seems unlikely to account for the change in properties. Rather, the variants could differ in the stability of configuration of cleaved VP₃ in the capsid. This hypothesis is supported by the finding here that the pattern of VP₃ cleavage by trypsin and crude trypsin was alike with SP and LP virus but that the associated antigenic changes differed. Also, VP₃ fragments obtained from trypsin-treated virus did not induce the formation of virus-neutralizing antibody seen with intact VP₃ (Bachrach et al. 1975). However, the same fragments contained in trypsin-treated virus capsids produced a neutralizing antibody response. Such conformational differences could account for the discrepancy in immunogenicity between trypsin-treated A₁₂ FMDV (Bachrach et al. 1975), C₃ FMDV (Rowlands et al. 1971) and O₁ FMDV (Wild & Brown, 1967; Strobbe et al. 1974).

The direct role of VP₃ in the infectivity and antigenicity of FMDV is supported by the present study. The fact that only VP₃ was cleaved by any of the enzyme treatments indicated the prominence of susceptible VP₃ peptide bonds. Iodination of intact virus was shown to label VP₃ (LaPorte & Lenoir, 1973; Bachrach et al. 1975). Here, VP₃ and the cleavage products of VP₃ were principally labelled in situ by ¹²⁵I; this result indicated that VP₃ plays a dominant role in the surface properties of intact and enzyme-treated particles as well. The other polypeptides must also be important in virus antigenicity, but probably indirectly. The role of VP₃ in immunogenicity has been suggested by changes associated with enzyme cleavage (Rowlands et al. 1971; Strobbe et al. 1974; Cavanagh et al. 1977) and by direct immunization with isolated virus polypeptides (LaPorte et al. 1973; Bachrach et al. 1975; Kaaden et al. 1977). Polypeptides VP₁ and VP₂ did not induce virus-neutralizing or precipitating antibody (Bachrach et al. 1975).

The importance of determining the properties of LP and SP variants of FMDV lies in their relationship to particular viruses causing foot-and-mouth disease in the field. Although properties and effects of enzymes on various serotypes of FMDV might be expected to vary, in this work, differences were shown for variants of the same serotype and subtype. In addition, antigenically different LP and SP variants were selectively produced on monolayer and suspension cell culture systems (Cowan et al. 1974; Meloen, 1976). Different cell line susceptibility (Cowan et al. 1974), pathogenicity, and tissue localization (McVicar & Sutmoller, 1972b) have been noted for variants of FMDV. Finally, and most important, the immune responses (Meloen, 1976) and protection in cattle to challenge with virulent FMDV (Cowan et al. 1974) differed with vaccines prepared with the virus variants. The differences listed above indicate the importance in understanding both the virus characteristics and cell system to be used in preparing effective vaccines for use in the field.

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REFERENCES


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