Structural Proteins of Foot-and-Mouth Disease Virus

By J. LAPORTE AND G. LENOIR
Station de Virologie et d'Immunologie, I.N.R.A.
78 Thiverval-Grignon, France

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SUMMARY

A mutant of the virus of foot-and-mouth disease (O type) was studied, and shown by electrophoresis in SDS-polyacrylamide gels to contain equimolar proportions of four polypeptides with mol. wts. of 34, 20, 17 and 14 × 10^3. Two minor components were also present with mol. wts. of 51 and 25 × 10^3 and in the molar ratio of 0.07 and 0.28. Only the polypeptide of mol. wt. 34 × 10^3 was labelled when the intact virus was iodinated. This polypeptide was sensitive to the action of trypsin and showed leucine as the N-terminal amino acid. Our own and other results are discussed in terms of virus structure.

INTRODUCTION

The number of polypeptides in foot-and-mouth disease virus has been the subject of considerable controversy during the last three years. Van de Woude & Bachrach (1968) considered that the multiplicity of bands obtained by polyacrylamide gel electrophoresis was due to aggregation of a single polypeptide. However, Wild, Burroughs & Brown (1969) used a mixture of [3H]-leucine and [14C]-threonine labelled viruses and showed that the multiple bands obtained by polyacrylamide gel electrophoresis were not due to aggregation. A recent paper by Van de Woude & Bachrach (1971) revised their earlier conclusion on the number of polypeptides in the virus.

Further evidence for the presence of more than one polypeptide in the virus was provided by our previous work which showed that threonine, leucine and isoleucine were present as N-terminal amino acids (Laporte, 1968, 1969). Burroughs et al. (1971) established definitely that foot-and-mouth disease virus, in common with other animal picornaviruses, contains several polypeptides.

This paper provides additional evidence for the existence of four to six polypeptides in a mutant of foot-and-mouth disease virus of O type, and relates these results to the model for mouse Elberfeld virus proposed by Rueckert, Dunker & Stoltzfus (1969).

METHODS

Virus. The virus used was a mutant of type O (Asso, 1967) obtained by cloning and selection at 29 °C. Virus was normally grown in monolayers of BHK 21 cells (MacPherson & Stoker, 1962) but labelled virus was grown in suspension cultures in a one-step growth curve. After infecting the cells at a multiplicity of 30 p.f.u./cell, and allowing adsorption to proceed for 45 min at 4 °C, the cells were washed and resuspended in Eagle's medium containing 2% calf serum at 36 °C to give a final concentration of 2 × 10^6 cells/ml. After
150 min 2 μCi/ml of [14C]-protein hydrolysate (C.E.A.) were added and the cells harvested after incubation for a further 150 min. The cells were then suspended in 0.16 M-tris-HCl buffer solution, pH 7.6, 2.5 × 10^{-3} M-EDTA, and the virus released by three successive freezings and thaws.

Labelled virus was purified by sedimentation in a 15 to 45 % sucrose gradient at 24000 rev/min for 4 h, using the SW 25 rotor of the L2 65B Spinco ultracentrifuge. The unlabelled virus was first concentrated by ultrafiltration on an XM 100 Diaflo membrane and two successive precipitations with 10 % polyethylene glycol. The concentrate was filtered through Sepharose 4 B in 0.16 M-tris-HCl buffer solution, pH 7.6, 2.5 × 10^{-3} M-EDTA, and the appropriate fractions mixed with caesium chloride to a density of 1.43 g/ml. The solution was then centrifuged for 18 h at 55000 rev/min in the SW 65 titanium rotor. The light scattering band was collected and examined in the electron microscope for the presence of virus particles.

Iodination of virus. The method described by MacFarlane (1958) and modified by Helm-kamp et al. (1960) was used. Stock iodine monochloride solution (0.02 M-ICl, 2.0 M-NaCl, 1 M-HCl) was shaken with carbon tetrachloride to remove free iodine and the traces of carbon tetrachloride then removed by passing a current of water-saturated air through the solution. One drop (0.05 ml) of the ICl solution was diluted to 2 ml with 2 M-NaCl and 1.25 ml of this solution plus 1 ml of [125I] (50 μCi) in 0.2 M-borate-NaOH buffer solution, pH 8.0, was added to 5 ml of purified virus in the same buffer solution. The virus was separated from free iodine by passing through a G-25 Sephadex column.

Polyacrylamide gel electrophoresis. The gels were prepared by the method of Summers, Maizel & Darnell (1965) except that ethylene diacrylate was used instead of N,N′-bis methylene acrylamide in order to make the gels soluble in ammonia. The gels contained 10 % acrylamide, 0.27 % ethylene diacrylate in 0.1 M-sodium phosphate buffer solution, pH 7.1, 0.5 M-urea and 0.1 % sodium dodecyl sulphate (SDS). Polymerization was achieved with 0.075 % ammonium persulphate and 0.5 % N,N,N′,N′-tetramethyl ethylene diamine.

All gels contained 10 % glycerol and measured 7 × 0.7 cm. Samples were allowed to migrate for 6 h at 8 mA/gel and 4 V/cm. For the preparations containing [14C], the gels were frozen and cut into 1 mm sections which were placed in 0.5 ml ammonia in counting vials and held at room temperature. After 2 h, 10 ml Bray’s scintillation fluid (Bray, 1960) was added prior to counting in a Packard spectrometer. The samples containing [125I] were counted directly in a Packard auto-gamma spectrometer.

The mol. wts. of the polypeptides were determined by comparison of their electrophoretic mobility on SDS gels with those of proteins with well established mol. wt. (Shapiro, Vinuela & Maizel, 1967). For this purpose rabbit globulins were mixed with the virus and disrupted as described above. The gels were then stained with Coomassie brilliant blue to ascertain the positions of the rabbit proteins before slicing and counting. Under these conditions (unpublished results) the rabbit globulins give light and heavy chains together with dimers of heavy chains. When plotted against migration distance the log (mol. wt.) of these three polypeptides were on a straight line.

Chromatography and dansylation. The methods described by Laporte (1969) were used except when 6 M-guanidine was used to disrupt the virus. In this case the polypeptides were separated by chromatography on columns of G-75 Sephadex equilibrated with 5 M-urea in 5 × 10^{-2} M-formic acid.
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Table 1

<table>
<thead>
<tr>
<th>Virus protein</th>
<th>Mol. wt.</th>
<th>Molar ratio</th>
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</thead>
<tbody>
<tr>
<td>VP 2</td>
<td>34000</td>
<td>1</td>
</tr>
<tr>
<td>VP 4</td>
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<tr>
<td>VP 5</td>
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<td>1</td>
</tr>
<tr>
<td>VP 6</td>
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<tr>
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<tr>
<td>VP 3</td>
<td>26000</td>
<td>0.28</td>
</tr>
<tr>
<td>VP' 2</td>
<td>42000</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Polyacrylamide gel electrophoresis of virus labelled with $^{14}$C-amino acids

Having shown by N-terminal determination that foot-and-mouth disease virus contained at least three polypeptide chains (Laporte, 1968, 1969) we attempted further characterization by SDS polyacrylamide gel electrophoresis. Fig. 1 shows the radioactivity profile of virus labelled by $^{14}$C-amino acids after disruption with 1% SDS, 0.5 M-urea followed by dialysis against 0.1 M-sodium phosphate buffer solution, pH 7.2, 0.5 M-urea, 0.1% SDS, 1% 2-mercaptoethanol. Usually 6 peaks were obtained (VP 1 to VP 6), but in some experiments a shoulder (VP' 2) appeared in front of VP 2.

The calculated mol. wt. of the virus proteins are given in Table 1. Similar results were obtained when virus samples were disrupted by heating at 100°C for 1 min or by treatment with 8 M-urea for 1 h.

From the mol. wt., the molar ratio of a component was determined as its proportion relative to the virus capsid. We attempted such measurements from the electrophoretic profile of disrupted virus labelled uniformly with a mixture of $^{14}$C-amino acids. The area of each peak was taken to be proportional to protein mass (Table 1). It appears that the four
higher peaks, VP 2, VP 4, VP 5 and VP 6 were present in approximately equimolar proportions; VP 1 and VP 3 were present in only small amounts.

**Polyacrylamide gel electrophoresis of iodinated virus**

Virus was iodinated by treatment with $[^{125}\text{I}]-\text{monochloride}$. The sedimentation coefficient of the iodinated virus was the same as that of untreated virus (Fig. 2) and its appearance in the electron microscope was unchanged. SDS polyacrylamide gel electrophoresis of iodinated virus (Fig. 3) showed that the radioactivity was located only in the VP 2 peak.

When iodination was performed after denaturation of the virus by 6 M-guanidine, the polyacrylamide gel pattern showed that each polypeptide was labelled (Fig. 4). Thus each polypeptide contained tyrosine but only in VP 2 was the tyrosine accessible for iodination in the intact virus.

**Location of the $[^{125}\text{I}]$ in the iodine-labelled virus**

Unlabelled virus was disrupted by 6 M-guanidine and chromatographed on G-75 Sephadex in $10^{-2}$ M-formic acid containing 5 M-urea. We established that the protein which was excluded from the gel had leucine as the N-terminal amino acid and that the fractions with lower mol. wt. had threonine and isoleucine. These results for N-terminal groups confirm previous results (Laporte, 1969).

When virus labelled with $[^{125}\text{I}]$ was examined by the same method, radioactivity was detected only in the protein excluded from the gel, showing that the iodine was present in the polypeptide with N-terminal leucine (Fig. 5).
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Fig. 3. (a) SDS-Polyacrylamide gel electrophoresis of polypeptides prepared from our O type mutant of foot-and-mouth disease virus, after labelling with \(^{125}\)I. (b) SDS-Polyacrylamide gel electrophoresis of \(^{14}C\)-amino acid labelled control. The gels were run in parallel.

This result was confirmed in an experiment in which iodinated virus was degraded by reacting with 8 M-urea in 0.02 M-tris–HCl, pH 8.6 buffer solution for 1 h, and then chromatographed on columns of DEAE-25 Sephadex in the same buffer solution. No radioactivity was adsorbed on the column and this excluded fraction contained leucine as the N-terminal amino acid (Fig. 6) (Laporte, 1969).

Wild et al. (1969) showed that the polypeptide on which trypsin acted was the slowest moving on electrophoresis in polyacrylamide gels, and Burroughs et al. (1971) confirmed this by electrophoresis on SDS polyacrylamide gels. We have shown that trypsin removed the label from iodinated virus and have confirmed that the polypeptide which is labelled is cleaved by trypsin.

DISCUSSION

These results provide new evidence that foot-and-mouth disease virus, like other animal picornaviruses, contains several different polypeptide chains.

Electrophoresis on SDS polyacrylamide gels has shown that the virus studied here is composed of six different polypeptide chains (Table 1). Four of the polypeptides: VP 2,
Fig. 4. SDS-Polyacrylamide gel electrophoresis of polypeptides prepared from our O type mutant of foot-and-mouth disease virus, after denaturation by 6 M-guanidine and labelling with $^{125}$I.

Fig. 5. Chromatography on columns of G-75 Sephadex in $10^{-5}$ M-formic acid, 5 M-urea of 6 M-guanidine disrupted virus. ■—■, $^{125}$I-labelled virus; ○—○, unlabelled virus trace at $E_{260}$. 
VP 4, VP 5, VP 6 are present in equimolar ratio, and this tetrameric subunit has a mol. wt. of about 85000.

Rueckert et al. (1969) suggested that the chemical, morphological and crystallographic structural units of mouse Elberfeld virus were identical and composed of three polypeptides with mol. wts. of about 30000. According to Caspar & Klug (1962) the capsid of the virus comprises 60 of these 'monomers'. Johnston & Martin (1971) and Talbot & Brown (1972) used the same model for bovine enterovirus and foot-and-mouth disease virus.

In the case of the mutant virus studied here, the 'monomer' is composed of four polypeptides and has a mol. wt. similar to that of the other picornaviruses (about 90000).

The following hypothesis may explain the difference in the number of polypeptides of the monomer. One of the three polypeptides, VP 2 described by Talbot & Brown (1972) (mol. wt. 30000) is cleaved once more to give our VP 5 and VP 6 (mol. wt. 17000 and 14000).

The role of VP 3 (Table I) is not clear. For 60 moles of VP 2, VP 4, VP 5 and VP 6 per virus particle, there would be 17 moles of VP 3 and the precision of our methods allows 20 or 12 for this number. In the first case VP 3 could play a role in the centre of each face of the icosahedron and in the second case it could be implicated in the structure of the 12 vertices.

In these cases the mol. wt. of the complete capsid would be \((85000 \times 60) + (26000 \times 20) = 5.6 \times 10^6\) or \((85000 \times 60) + (26000 \times 12) = 5.4 \times 10^6\). Both numbers are in accord with the estimate of Bachrach, Trautman & Breese (1964).

The virus protein VP 1 may correspond with the polypeptide \(\epsilon\) of mouse Elberfeld virus. Iodination of the intact virus showed that only one polypeptide chain was labelled. This polypeptide (VP 2) had a mol. wt. of 34000 and contained leucine as the \(N\)-terminal amino
acid. Iodinated VP 2 was removed by incubation of the virus with trypsin and suggested its identity with the protein which carries the immunizing activity of the virus (Wild et al. 1969).

The differences between our results and those of Talbot & Brown (1972) concerning the number of polypeptide chains in the capsid of foot-and-mouth disease virus are not due to technical differences because our results are unchanged when we use their procedures. The sum of the mol. wts. of the three polypeptides obtained by these authors is the same as the sum of the mol. wts. of the four polypeptides in this study. We are investigating this as the possible weakness of the capsid of our mutant virus.

REFERENCES


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