A Model for Foot-and-Mouth Disease Virus

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The protein composition of several members of the animal picornavirus group is remarkably similar. Thus, the enteroviruses polio type I, Echo 12 and bovine enterovirus VG-5-27 (Maizel & Summers, 1968; Korant, Lonberg-Holm & Halperen, 1970; Johnston & Martin, 1971), the cardioviruses Maus-Elberfeld and encephalomyocarditis (Rueckert, Dunker & Stoltzfus, 1969; A. T. H. Burness, personal communication), the rhinoviruses 1A and HGP (Medappa, McLean & Rueckert, 1971; E. J. Stott & R. Killington, personal communication) and several of the seven immunological types of foot-and-mouth disease virus (Burroughs et al. 1971; P. Talbot, unpublished observations) contain three polypeptides with molecular weights ranging from 37 to 24 × 10^3 (VP1, VP2, VP3) and a smaller polypeptide with a molecular weight in the range of 7 to 13.5 × 10^3 (VP4). In several instances the molar ratio of the four polypeptides has been determined and, with the exception of rhinovirus 1A (Medappa et al. 1971), the three larger polypeptides are present in equimolar amounts and the smaller polypeptide is present in a half molar amount relative to the others. In the case of rhinovirus 1A, all four polypeptides were reported to be present in equimolar amounts (Medappa et al. 1971). However, E. J. Stott & R. Killington (personal communication) have shown that the polypeptide composition of the HGP strain of rhinovirus is similar to that of the other picornaviruses listed above.

The only data which differ from this general pattern have been provided by Korant et al. (1970) for two rhinoviruses and Mak et al. (1971) for variants of Mengo virus. These authors found only three peaks by polyacrylamide gel electrophoresis. K. K. Lonberg-Holm (personal communication) has since found a fourth polypeptide in the rhinoviruses and it seems probable from the molecular proportions of VP1 and VP2 in Mengo virus (Maket al. 1971) that VP1 contains two polypeptides of the same molecular weight. In addition, Rueckert et al. (1969) and Medappa et al. (1971) have detected small amounts of a larger polypeptide (molecular weight 41 × 10^3) in Maus-Elberfeld virus and rhinovirus 1A which they consider to be an uncleaved precursor of two of the virus polypeptides.

Rueckert et al. (1969) suggested a model for Maus-Elberfeld virus which was based on the proposals made by Finch & Klug (1966) for the capsid structure of turnip yellow mosaic virus. This structure exhibited icosahedral symmetry and was composed of 180 identical polypeptides. In the Rueckert model for Maus-Elberfeld virus, the capsid consisted of 60 monomeric structures, each containing equimolecular amounts of the three larger polypeptides α, β and γ. The monomeric units were held together by two sets of bonds. One set bonded the monomeric units into pentamers and the second set held 12 of the pentamers together to form a shell of 60 monomeric units.

Incubation of Maus-Elberfeld virus at pH 5.7 in the presence of 0.1 M-sodium chloride for 1 hr at 37° specifically disrupted the bond which held the pentamers together, releasing the virus RNA, the 12 pentamers (as a 14S sub-unit containing only polypeptides α, β and γ) and an aggregate which consisted of the fourth polypeptide, δ. Mengo virus and encephalomyocarditis virus, which are closely related to Maus-Elberfeld virus, can also be dissociated into similar units by the same method (O'Callaghan, Mak & Colter, 1970; A. T. H. Burness, personal communication).
Fig. 1. Polyacrylamide gel electrophoresis of foot-and-mouth disease virus polypeptides prepared from virus labelled with \([^{14}C]\)-amino acids.

Table 1. Calculation of molar ratios of the polypeptides in foot-and-mouth disease virus

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method of dissociation</th>
<th>Polypeptide</th>
<th>Molecular weight ( \times 10^{-3} )</th>
<th>Radioactivity (%)</th>
<th>Molar ratio</th>
<th>Polypeptides per virus particle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>8 M-urea</td>
<td>VP₁</td>
<td>34</td>
<td>35·1</td>
<td>1·03</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1 % SDS</td>
<td>VP₂</td>
<td>36</td>
<td>31·0</td>
<td>1·03</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1 % mercaptoethanol</td>
<td>VP₃</td>
<td>26</td>
<td>26·9</td>
<td>1·04</td>
<td>60</td>
</tr>
<tr>
<td>(see Fig. 1)</td>
<td></td>
<td>VP₄</td>
<td>13·5</td>
<td>7·0</td>
<td>0·52</td>
<td>30</td>
</tr>
<tr>
<td>Virus</td>
<td>0·5 M-urea</td>
<td>VP₁ + VP₂</td>
<td>64</td>
<td>66·1</td>
<td>1·03</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1 % SDS</td>
<td>VP₃</td>
<td>26</td>
<td>27·0</td>
<td>1·04</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1 % mercaptoethanol</td>
<td>VP₄</td>
<td>13·5</td>
<td>6·9</td>
<td>0·51</td>
<td>30</td>
</tr>
<tr>
<td>(see Fig. 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin-treated</td>
<td>0·5 M-urea</td>
<td>Modified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>virus</td>
<td>1 % SDS</td>
<td>VP₁ + VP₂</td>
<td>50</td>
<td>59·9</td>
<td>1·20</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>1 % mercaptoethanol</td>
<td>VP₃</td>
<td>26</td>
<td>31·8</td>
<td>1·22</td>
<td>61</td>
</tr>
<tr>
<td>(see Fig. 6)</td>
<td></td>
<td>VP₄</td>
<td>13·5</td>
<td>8·1</td>
<td>0·60</td>
<td>30</td>
</tr>
</tbody>
</table>

Analysis by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate shows that foot-and-mouth disease virus (type O) contains four polypeptides (VP₁ to VP₄) with molecular weights of 34, 30, 26 and 13·5 \( \times 10^3 \) (Fig. 1). These polypeptides are present in the ratio 1:1:1:0·5 (Table 1), in agreement with the proportions found for several of the picornaviruses referred to above. Foot-and-mouth disease virus can be dissociated at pH 6·5 into the free RNA, a protein sub-unit consisting of the three larger polypeptides and an insoluble precipitate containing the smallest polypeptide (Burroughs et al. 1971). In contrast to the sub-unit obtained from Maus-Elberfeld virus which sediments at 14S,
I 10 15 20 25 30

\[ \text{Fraction} \]

Fig. 2. Sucrose density gradient centrifugation of the 14s sub-unit from \([^{14}\text{C}]\)-amino acid labelled encephalomyocarditis virus and the 12s sub-unit from \([^{3}\text{H}]\)-amino acid labelled foot-and-mouth disease virus. The mixture of sub-units was centrifuged in one tube for 18 hr at 30,000 rev./min. in a 15 to 25% sucrose gradient in the MSE rotor No. 2418. • —— \([^{14}\text{C}]\); ○ —— ○, \([^{3}\text{H}]\).

however, the sub-unit from foot-and-mouth sedimented at about 12s in sucrose gradients. Independent observations made over a long period have given values ranging from 11.8 to 14s for the foot-and-mouth disease virus sub-unit (Trautman, Savan & Breese, 1959; Liebermann & Gralheer, 1968; J. N. Burroughs, personal communication).

We have now obtained direct evidence that the sub-unit from foot-and-mouth disease virus has a lower sedimentation coefficient than the sub-unit from encephalomyocarditis virus by co-sedimenting the sub-units in the same sucrose gradient. Foot-and-mouth disease virus labelled with \([^{3}\text{H}]\)-amino acids was adjusted to pH 6.5 with 0.05M-sodium dihydrogen phosphate and mixed with encephalomyocarditis virus labelled with \([^{14}\text{C}]\)-amino acids which had been incubated at 37° for 1 hr in 0.1M-sodium chloride, 0.1M-phosphate, pH 5.7. The mixture was then centrifuged in a 15 to 25% sucrose gradient in 0.04M-phosphate for 18 hr at 30,000 rev./min. in an MSE 3 × 20 ml. swing out rotor No. 2418 (mean centrifugal force = 75,000g). The distribution of radioactivity in the tube clearly demonstrates the different sedimentation rates of the two sub-units (Fig. 2). Accepting the value of 14s for the sub-unit of encephalomyocarditis virus, the sub-unit of foot-and-mouth disease virus has a sedimentation coefficient of 12s.

Strohmaier (1972) and Liebermann & Schulze (1971) estimated the molecular weight of the 12s sub-unit of foot-and-mouth disease virus to be \(289 \times 10^3\) and \(282 \times 10^3\) respectively from its sedimentation coefficient and diffusion constant. Using the method described by Hedrick & Smith (1968), the mobility of the 12s sub-unit through non-denaturing polyacrylamide gels with concentrations ranging from 4 to 7.5% was compared with the mobilities of the monomer, dimer, trimer and tetramer of bovine plasma albumin in the same gels (Fig. 3a). This method gave a value of \(265 \times 10^3\) for the molecular weight of the 12s sub-unit (Fig. 3b). Supporting evidence for this value was obtained from similar
estimates of the molecular weight of the protein sub-unit obtained by pH 6·5 disruption of trypsin-treated virus. Treatment of the virus with this enzyme does not affect the morphology of the virus particles but the infectivity is greatly reduced because part of the polypeptide VP1, which attaches to the cell receptor sites is digested by the trypsin (Burroughs et al. 1971). The molecular weight of the modified VP1 is \(19 \times 10^3\), a reduction of \(15 \times 10^3\) compared with the molecular weight of VP1. The molecular weight of the protein sub-unit produced by pH 6·5 treatment of the trypsin-treated virus was found to be \(220 \times 10^3\) (Fig. 3b) which is \(45 \times 10^3\) less than that of the 12s sub-unit produced by similar treatment of the intact virus. This reduction in molecular weight indicates that there are three molecules of VP1 in the 12s sub-unit. The molecular weight of the 12s sub-unit is thus considerably less than the values of \(420\) and \(410 \times 10^3\) given by Rueckert et al. (1969) and Mak et al. (1970) for the 14s sub-units of Maus-Elberfeld and Mengo viruses respectively.

The molecular weights of the three proteins comprising the 12s sub-unit of foot-and-mouth disease virus are 34, 30 and \(26 \times 10^3\) and, as they are present in equimolar proportions, one monomeric unit will have a molecular weight of \(90 \times 10^3\). This is compatible with the 12s sub-unit being a trimer with a molecular weight of \(270 \times 10^3\).

The molecular weight of foot-and-mouth disease virus RNA has been variously estimated...
Fig. 4. Diagrammatic representation of the dissociation of the icosahedral structure of encephalomycocarditis virus and foot-and-mouth disease virus into 14s and 12s sub-units respectively showing the locations of VP₁ to VP₄ as 1, 2, 3, 4.

To be 2.0 to 3.1 x 10⁶ (Strohmaier & Mussgay, 1959; Wild & Brown, 1970; Liebermann & Schulze, 1971). Using the method described by Fenwick (1968) in which the RNA is treated with formaldehyde to minimize configurational restraints before centrifuging in sucrose gradients, J. F. E. Newman (personal communication) has found from co-sedimentation experiments that the sedimentation coefficient of the RNA is the same as that of encephalomycocarditis virus RNA. Values of 2.4 x 10⁶ (Fenwick, 1968) and 2.7 x 10⁶ (Burness & Clothier, 1970) have been obtained for the molecular weight of encephalomycocarditis virus RNA by physical and chemical methods. Taken in conjunction with the value of 2.6 x 10⁶ for poliovirus RNA obtained by electron microscopy (Granboulan & Girard, 1969) and by physical methods (Tannock, Gibbs & Cooper, 1970) it seemed reasonable to use a value of 2.6 x 10⁶ for the molecular weight of foot-and-mouth disease virus RNA in our calculations. Since the RNA comprises 31% of the virus particle (Bachrach, Trautman & Breese, 1964), the molecular weight of the virus is 2.6 x 10⁶ x 100/31 = 8.4 x 10⁶, of which the protein will contribute 5.8 x 10⁶. The value for the molecular weight of the virus is similar to those given by Scraba, Kay & Colter (1967) and Burness & Clothier (1970) for Mengo and encephalomycocarditis viruses respectively but is considerably greater than that given by Liebermann & Schulze (1971) for foot-and-mouth disease virus.

A molecular weight for foot-and-mouth disease virus protein of 5.8 x 10⁶ would be in
Short communications

Fig. 5. Polyacrylamide gel co-electrophoresis of \([^{14}C]\)-amino acid labelled foot-and-mouth disease virus disrupted with 1 \(\%\) sodium dodecyl sulphate, 1 \(\%\) mercaptoethanol and 0.5M-urea, and \([^{35}S]\)-amino acid labelled virus disrupted with 1 \(\%\) sodium dodecyl sulphate, 1 \(\%\) mercaptoethanol and 8M-urea. ● ——●, \([^{14}C]\); ○ ——○, \([^{35}S]\).

accordance with the presence of twenty 12S sub-units (total molecular weight 5.4 \(\times\) 10^6) and 30 molecules of VP4 (total molecular weight 30 \(\times\) 13.5 \(\times\) 10^3 = 0.41 \(\times\) 10^6) in each virus particle. The 12S sub-units represent the 20 triangular faces of the virus particle but the role of VP4 is not known with any certainty. Johnston & Martin (1971) have proposed a location for VP4 in the structurally related bovine enterovirus VG-5-27 which is based on the argument that the 30 chains of the polypeptide should be located in 30 identical domains. The only location in the Rueckert model which satisfies this requirement is at the apposition of the faces of the icosahedron through which the two fold axes of symmetry pass (Fig. 4). The molar ratio of VP4 in Maus-Elberfeld and foot-and-mouth disease viruses suggests a similar location for the polypeptide in these viruses. This arrangement would have the added advantage that VP4 would be at a position from which it could be released when the 14S and 12S sub-units are dissociated from the two viruses respectively (Fig. 4).

Resolution of the fine structure of the protein shell of these viruses will require alternative methods for disrupting the particles. In preliminary experiments we have shown by polyacrylamide gel electrophoresis that foot-and-mouth disease virus can be disrupted with 0.5M-urea, 1 \(\%\) sodium dodecyl sulphate and 1 \(\%\) mercaptoethanol at 37\(^\circ\) into polypeptides with molecular weights of 64, 26 and 13.5 \(\times\) 10^3 (Fig. 5). The components with molecular weights of 26 and 13.5 \(\times\) 10^3 correspond to VP3 and VP4 obtained by disruption with 8M-urea (Burroughs et al. 1971) and the component with molecular weight 64 \(\times\) 10^3 apparently consists of one molecule each of VP2 and VP3. Supporting evidence for this view was obtained from similar experiments with trypsin-treated virus. In this case, components with molecular weights of 50, 26 and 13.5 \(\times\) 10^3 were obtained (Fig. 6), the 50 \(\times\) 10^3 component
corresponding to one molecule of VP₂ and one molecule of trypsin-modified VP₁. This intermediate cleavage of the virus protein shell suggests that the bonds between VP₁ and VP₂ are stronger in 0.5M-urea, 1% sodium dodecyl sulphate and 1% mercaptoethanol than those between VP₁ and VP₃ on the one hand and VP₂ and VP₃ on the other.

The Rueckert model for Maus-Elberfeld virus can account for the observations made with foot-and-mouth disease virus. With the latter virus dissociation at pH 6.5 is regarded as being caused by breakage of the protein–protein bonds along the edges of the triangular faces, in contrast to Maus-Elberfeld virus where the bonds break so as to yield a pentamer or 14s sub-unit. Although Rueckert et al. (1969) and Johnston & Martin (1970) were unable to make specific assignments for the positions of the three larger polypeptides, evidence from electron microscopy of complexes of foot-and-mouth disease virus and antibody indicates that the binding site removed by trypsin is at the vertices of the icosahedron (Brown & Smale, 1970). Since we have already shown (Burroughs et al. 1971) that VP₁ is the only polypeptide of the virus which is modified by the enzyme, it would appear that VP₁ is located at the vertices of the icosahedron.

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REFERENCES


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