The Structure of Foot-and-Mouth Disease Virus Protein

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Several picornaviruses appear to contain more than one polypeptide chain. When examined by polyacrylamide gel electrophoresis, poliovirus (Maizel, 1963; Maizel & Summers, 1968) and mouse encephalomyocarditis virus (Burness & Walter, 1967), each gave four bands and mouse encephalitis virus gave three bands (Rueckert & Duesberg, 1966). The most reasonable explanation for these observations is that each of the viruses contains more than one polypeptide chain; but the multiplicity of the bands could be due to chemical modification of certain amino acids in the virus protein during the experiment or to aggregation or breakdown of a single protein. Vande Woude & Bachrach (1968) considered that the multiplicity of bands they obtained from foot-and-mouth disease virus was due to aggregation of a single polypeptide. However, Wild, Burroughs & Brown (1969) used a double labelling technique and concluded that the multiple bands obtained with this virus are not due to aggregation.
of a single polypeptide chain. I present here chemical evidence that the protein of foot-and-mouth disease virus contains at least three distinct polypeptide chains.

A heat stable mutant of type O virus (Asso, 1967) was grown in monolayers of BHK 21 cells which had been produced in rotating 11. bottles. The virus was purified according to the method described by Brown & Cartwright (1963) except that the concentrated virus was first chromatographed on Sepharose 2B before centrifugation in a sucrose gradient. Purified virus was suspended in 0.02 M-tris + HCl buffer, pH 8.6,

![Fig. 2. Chromatography on DEAE-Sephadex of foot-and-mouth disease virus which had been degraded with 8 M-urea at pH 8.6. ×—×, Absorbance at 260 nm.; ○——○, absorbence at 280 nm.; ..., molarity of NaCl.](image)

adjusted to pH 10.5 and reacted with 8 M-urea for 16 hr at 40° (Boeyé & van Elsen 1967). After dialysis against distilled water it was dansylated with dansyl chloride (Gray, 1967). Specific identification of the residues was made by two-dimensional thin-layer chromatography on silica gel or by chromatography on polyamide paper following hydrolysis of the dansylated product with 6 N-HCl for 16 hr at 100°. For the first dimension 1.5% formic acid in water was used and n-butanol:n-heptane: glacial acetic acid (6:6:2, v/v) was used for the second dimension. Three spots were obtained, corresponding to the dansylated derivatives of leucine, iso-leucine and threonine (Fig. 1).

The virus was also degraded in two stages by reacting it with 8 M-urea, first at pH 8.6 and then at pH 10.5. After treating with 8 M-urea in 0.02 M-tris + HCl, pH 8.6, for 1 hr, the degraded virus was chromatographed on a DEAE Sephade X A-25 column in
equilibrium with the same buffer. A peak of protein (A) passed unadsorbed through the column and the remaining part of the virus (B) was eluted by applying a gradient of sodium chloride (Fig. 2). Fraction B was dialysed against 0.02 M-tris + HCl, pH 8.6, made 8 M with respect to urea and the solution adjusted to pH 10.5 with 0.1 N-NaOH. After 16 hr at 40º, this solution was chromatographed on DEAE-Sephadex A-25. As in the first chromatogram, protein (peak C) passed unadsorbed through the column and a peak (D) of RNA was obtained when a gradient of sodium chloride was applied (Fig. 3). Each protein fraction was dansylated and the products examined as described above. Peak A gave dansyl-leucine only and peak C gave dansyl-iso-leucine and dansyl-threonine.

These results provide chemical evidence that foot-and-mouth disease virus contains at least three polypeptide chains. They also show that one of the polypeptides is less firmly bound to the RNA of the virus than are the other two polypeptides.

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