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The Positively Charged Structural Virus Protein (VP_1) of Foot-and-Mouth Disease Virus (Type O_1) Contains a Highly Basic Part which may be Involved in Early Virus–Cell Interaction

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SUMMARY

Polypeptides of 'trypsin-resistant' (TR) variants of foot-and-mouth disease virus type O_1 (BFS 1860) were analysed by electrofocusing and two-dimensional gel electrophoresis. In contrast to parent O_1 virus, trypsin treatment of these variants did not reduce their infectivity and their ability to attach to susceptible cells, although VP_1 was cleaved as in the parent virus. In OTR_1, one of the cloned isolates, an additional polypeptide (VP_A) with a mol. wt. approx. 31×10^3 (31K), was found which resembled VP_1 (28K) in being positively charged and cleaved by trypsinization of the virus into a neutral 18K polypeptide (P18) and a strongly basic fragment (pI > 10) with a mol. wt. of approx. 6K (P6). These findings substantiate the hypothesis that VP_A is an elongated VP_1. While P18 fragments of both trypsin-treated parent virus and OTR progeny viruses focused at identical (neutral) pH, P6 fragments of trypsinized OTR variants (including OTR_1) were even more positively charged than P6 of parent virus. This difference in charge of the P6 polypeptide may be responsible for the retained cell attachment ability of trypsinized OTR viruses. The data are discussed with respect to the known amino acid sequence of VP_1 of the closely related O_1 Kaufbeuren.

In a previous paper we described the isolation and characterization of foot-and-mouth disease virus (FMDV) variants which showed no significant loss of infectivity upon trypsinization (Barteling *et al.*, 1979). These 'trypsin-resistant' (TR) viruses were obtained by serial passage in BHK cells of virus that had been treated with trypsin before inoculation of the cells.

In contrast to trypsin-treated parent virus (ttO), which does not adsorb to susceptible cells (Brown *et al.*, 1963; Rowlands *et al.*, 1971), trypsin-treated TR virus (ttOTR) still attached to BHK cells (Barteling *et al.*, 1979). Although some differences could be observed between untreated parent O virus and its 'trypsin-resistant' progenies in immunodiffusion tests and by SDS–polyacrylamide gel electrophoretic (SDS–PAGE) analysis with one of the variants, after trypsinization these disappeared. The only property that could be correlated with retained infectivity of ttOTR was a less-negative charge than ttO (Barteling *et al.*, 1979). TR virus could also be isolated directly by electrophoresis of (uncloned) ttO, especially from the area representing the typical mobility of ttOTR (S. J. Barteling *et al.*, unpublished data). Thus, retained infectivity after trypsinization seems to be associated with a characteristic electrical charge of ttOTR.

We tried to detect the basis of the difference in charge between ttO and ttOTR variants by electrofocusing and two-dimensional (2D) analysis (O'Farrell *et al.*, 1977) of the virus proteins. Electrofocusing was done on a cooled flat bed electrophoresis apparatus. Gel slabs of 1 mm thick were poured between two parallel glass plates as described by Kerckaert (1978). The gel mixture was composed of 8·5 M-urea, 2% Nonidet P40 (NP40), 4% acrylamide/bisacrylamide (18:1) and 2% ampholine pH 3 to 10. Purified virus was disrupted

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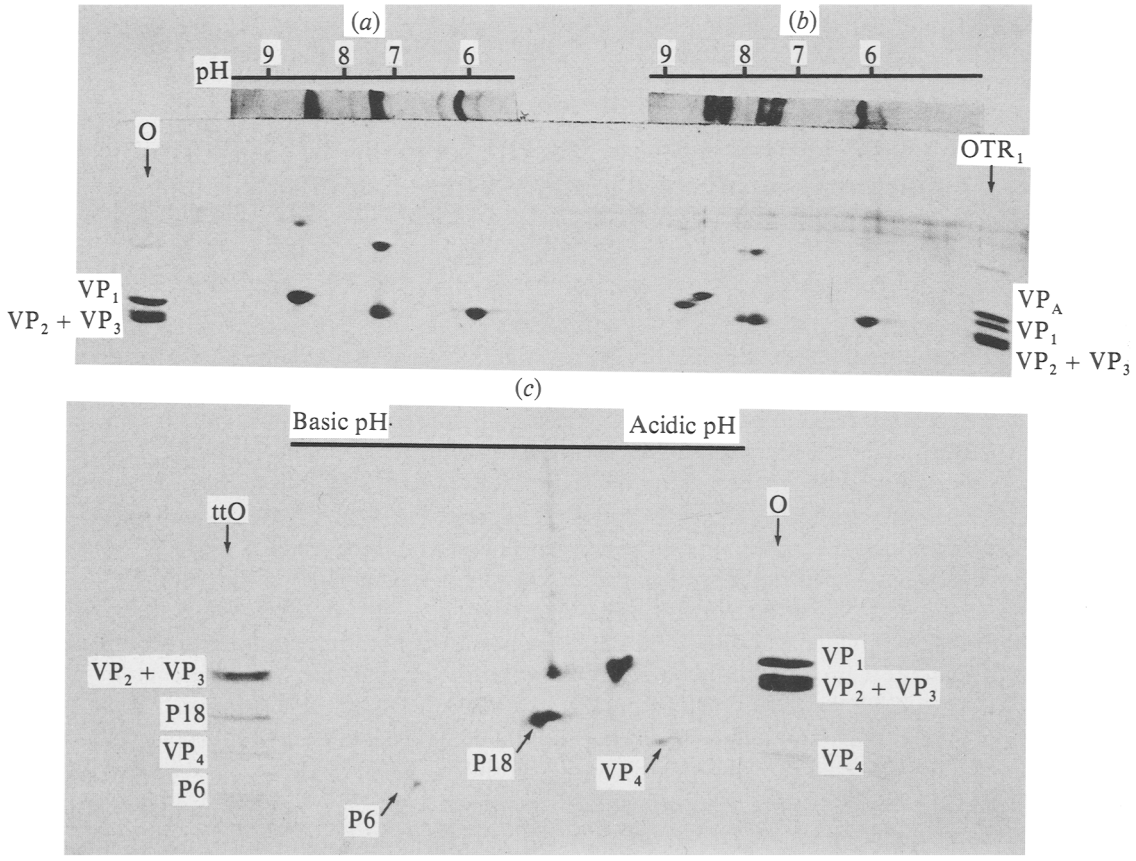

Fig. 1. Two-dimensional analysis of (*a*) untreated O, (*b*) untreated OTR_1 and (*c*) trypsin-treated O (ttO). The first dimension electrofocusing was for 90 min (*a*, *b*) and 45 min (*c*) at 400 V. Reference preparations for the second (SDS–PAGE) dimension were O (*a*), OTR_1 (*b*) and O and ttO (*c*).

in sample buffer (9·5 M-urea, 2% NP40, 2% ampholine, 5%-β-mercaptoethanol and a trace of crystal violet). After 15 min at room temperature the sample was added to the wells. Electrophoresis was started at 100 V for 30 min and continued for 45 min or longer at 400 V and 22 °C. Ampholine was removed from the gel by washing in 30% methoxyethanol brought to pH 9 with Tris. This prevented elution of small basic fragments from the gel. Electrofocusing gels were stained by addition of Coomassie Brilliant Blue to the methoxyethanol. For the second dimension in the 2D analysis, 4 mm-wide strips were cut from the gel, placed on top of the SDS–PAGE gel and embedded in 1% agarose in SDS sample buffer. The discontinuous buffer system of Laemmli (1970) was used with some modifications (Barteling *et al.*, 1979).

In a previous study it was shown that one of the TR clones (OTR_1) contained an additional virus protein (VP_A), about 3K larger than VP_1 of this clone. About 50% of VP_1 was replaced by VP_A. Trypsinization of OTR_1 resulted in the cleavage of VP_A and VP_1 into similar main fragments (18K and 6K). Also, iodination experiments showed that, like VP_1, VP_A is located at the surface of the virus particle (Barteling *et al.*, 1979). In addition, 2D analysis (Fig. 1*b*) showed that, like VP_1 (pI 8·7), VP_A is basic (pI 8·5). It has also been shown by others that VP_1 has a positive charge (Crowther, 1977; Robson *et al.*, 1979). The P18 fragments of both VP_1 and VP_A have the same charge and chymotrypsinization of parent virus and of OTR_1 cleaved VP_A and VP_1 into main fragments of 20K and 8K (data not shown). Thus, VP_A

seems to be an elongated VP_1. The presence of VP_A is probably caused by an alternative cleavage site in the precursor polyprotein. If the elongation was at the N-terminus of VP_1 we should find a shorter VP_3, the virus protein adjacent to the N-terminal end of VP_1 in the polyprotein (Sangar *et al.*, 1977). This was not the case and so the elongation is probably at the C-terminal side in the non-structural part of the polyprotein. The additional oligopeptide fragment seems to cause the slightly reduced positive charge of VP_A with respect to VP_1. This is in accordance with the amino acid sequence deduced from cDNA studies of O_1 Kaufbeuren, a closely related O_1 isolate (Kurz *et al.*, 1981). If elongation of VP_1 occurred by extension through the normal cleavage site into the N-terminal region of the adjacent non-structural protein for approx. 25 residues, the extra protein fragment would contain an excess of negative over positive amino acids. The C-terminal leucine (coded by UUG) is followed by asn-phe. An identical C-terminal cleavage site of VP_1 is also found in A_{12} (Kleid *et al.*, 1981). Twenty-five amino acids downstream in the non-structural part of the polyprotein of O_1 virus can be found another asn-phe sequence, this time preceded by serine (coded by UCG). If by a point mutation the cytosine is by deamination transformed into a uridine, which seems to happen quite frequently in nature, this would change serine into leucine and may create an alternative cleavage site resulting in an approx. 3K larger VP_1 molecule.

As already mentioned, in ttO the basic virus protein VP_1 is found to be cleaved into main fragments of 18K and 6K. By 2D analysis P18 was detected at near neutral pH while under our original conditions, electrofocusing for 2·5 h, no P6 spot was found (data not shown). The neutral character of P18 suggested a highly positive charge for the other cleavage products of the basic VP_1. Thus, P6 could well disappear at the cathode during electrofocusing. If electrofocusing was applied for only 45 min a fragment could be detected ahead of VP_1 and ahead of the crystal violet marker (c.v.), which has a pI of 9·1 (Fig. 2). By 2D analysis this fragment was shown to be P6 (Fig. 1*c*). If electrofocusing was performed for more than 60 min this fragment left the gel slab, showing that it has a pI above 10, the highest pH in our slabs. VP_1 of O_1 virus contains 34 positively charged compared to 17 negatively charged amino acids (Kurz *et al.*, 1981), explaining the basic nature of VP_1. According to data obtained by Strohmaier *et al.* (1982) P18 and P6 of this virus have a surplus of respectively three and nine positively charged amino acids which, in relation to their molecular weights, may explain the pI of approx. 7·7 and above 10 as found here.

The main virus proteins often appeared partially as polymers in our 2D gels (Fig. 1) for which we have no explanation. VP_1 of OTR_2 and OTR_3 was more basic than VP_1 of parent virus as shown by electrofocusing for 2·5 h at 400 V (Fig. 2, inset). The other virus proteins of the TR variants focused at the same positions as those of parent virus. No difference was observed between the isoelectric points of VP_1 of OTR_1 and of parent virus. After trypsinization the P18 fragments of parent virus and all OTR variants focused at an identical pH; however, P6 fragments of ttOTR viruses (including OTR_1) were found at an even higher pH than P6 of ttO (Fig. 2). This higher pI of P6 fragments of ttOTR variants could explain the higher charge of VP_1 of OTR_2 and OTR_3. One explanation for the identical charge of VP_1 of OTR_1 to VP_1 of parent virus could be a (second) mutation in the part(s) of VP_1 that is removed by trypsinization. This mutation should then neutralize the effect of the added positive charge in P6. The higher charge of the P6 fragment might also explain why ttOTR variants remain more positively charged that ttO and, since this higher charge is likely to be associated with retained cell attachment ability of ttOTR viruses, P6 may play a role in this respect.

Why P6, which contains a number of arginine and lysine molecules, is not cleaved further by trypsin is not clear. If P6 of OTR variants plays a role in retained attachment of these viruses after trypsinization, this part of VP_1 must at least partly be located superficially. This

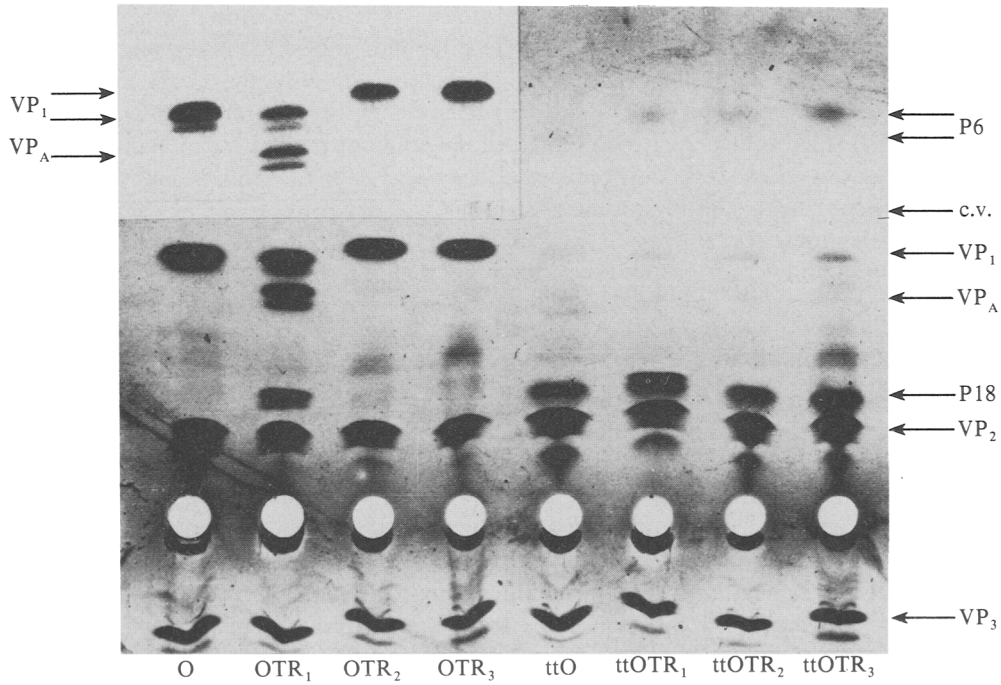

Fig. 2. Electrofocusing (45 min at 400 V) of O_1 parent virus and of 'trypsin-resistant' progeny viruses OTR_1, OTR_2 and OTR_3 before and after trypsin treatment (tt). Inset shows relative positions of VP_1 after 2·5 h electrofocusing. The P18 (or P20) fragment of untreated OTR_1 is not characteristic for this variant and occasionally occurs in preparations of parent (O) virus also. c.v., Crystal violet marker (pI 9·1).

may be in a surface pocket as has recently been suggested for the receptor interaction site of the haemagglutinin of influenza virus (Wilson *et al.*, 1980). Location in such a pocket-like structure may protect P6 from digestion by trypsin. Since all FMDV strains studied so far have a basic VP_1 it is very probable that these VP_1 molecules all contain such a highly basic part. This phenomenon may be common in picornaviruses in general since poliovirus also contains a basic VP_1 molecule (Hamann *et al.*, 1977). In this respect it is striking that among the 60 C-terminal amino acids of FMDV type A_{12} there are 13 positive compared to five negatively charged residues (Kleid *et al.*, 1981) and in poliovirus type 1 this balance is 13 compared to four (Kitamura *et al.*, 1981). It is tempting to speculate that this part of VP_1 might have an identical function in picornaviruses.

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