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DETECTION OF T1-OLIGONUCLEOTIDES OF THE FOOT-AND-MOUTH DISEASE VIRUS DNA BY SILVER STAINING

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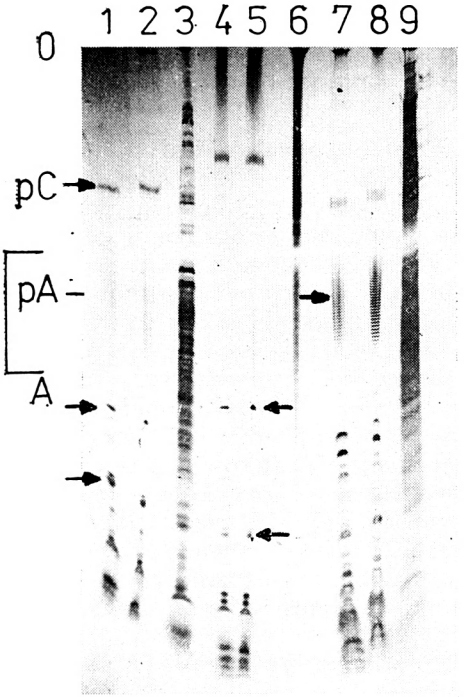
Summary. — Viral (v) RNA was isolated from purified foot-and-mouth disease virus (FMDV) by phenol-chloroform-isoamylalcohol treatment, digested by RNase T1 and separated by one-dimensional polyacrylamide gel electrophoresis (PAGE). The oligonucleotides were detected by silver staining. About 45 μg of vRNA corresponding to about 100 ml of infectious BHK-21 cell culture fluid yielded a pattern of nearly 20 bands sufficient to differentiate between the FMD viruses.

Oligonucleotide mapping of picornavirus RNAs, as described by Frisby *et al.* (1976) and Harris and Brown (1977) and optimized by La Torre *et al.* (1982) was used progressively in the last years to characterize and differentiate between virus strains (Smith and Lombard, 1984; Anderson *et al.*, 1985; Hamblin *et al.*, 1985; Sobrino *et al.*, 1986; Giraudo *et al.*, 1987; and others). The method exploits the high specificity of the RNase T1 which cleaves the RNA at the guanyl acid residues. As a rule, the labelled RNA fragments of different sizes are separated by one- or two-dimensional electrophoresis in PAG and then visualized by autoradiography. The oligonucleotide maps are suitable for differentiation of the even closely related strains. In our experiments we tested the applicability of silver staining for the identification of oligonucleotides of FMDV RNA avoiding problems associated with radioactive labelling. Our first results using preliminary one-dimensional PAGE are reported.

The vRNA was obtained from fresh BHK-21 cells concentrated by PEG 6000 and purified by density gradient centrifugation (DGC), after phenol-chloroform-isoamylalcohol treatment, the vRNA was analysed by SDS-sucrose-DGC (Liebermann *et al.*, 1987). Following alcohol precipitation, the RNA was dissolved in T1-buffer [0.02 mol/l Tris-HCl, 0.02 mol/l dinatriumdihydrogenethylendiamintetraacetat (EDTA) pH 7.6]; and treated with RNase T1 (Serva, Heidelberg or Boehringer, Mannheim, F.R.G.) for 1 hr at 37 °C. Then 3 μl RNase (90— 300 U) were added to 6— 12 μl vRNA solution containing about 50 μg of vRNA. After addition of bromophenol blue dye marker and RNase free sucrose the T1-resistent oligonucleotides were separated by one-dimensional PAGE (gel size 360 × 200 × 1 mm). Marker was allowed to run for about 25 cm in a buffer containing 6 mol/l urea, 0.1 mol/l Tris-borate, 0.002 mol/l EDTA pH 8.3. The silver staining was carried out as described for rotavirus-RNA (Herring *et al.*, 1982). The gel was rinsed with distilled water, placed into a flat transparent plastic container and gently shaken in 10% ethanol and 5% acetic acid for 30 min. Thereafter, the gel was washed briefly



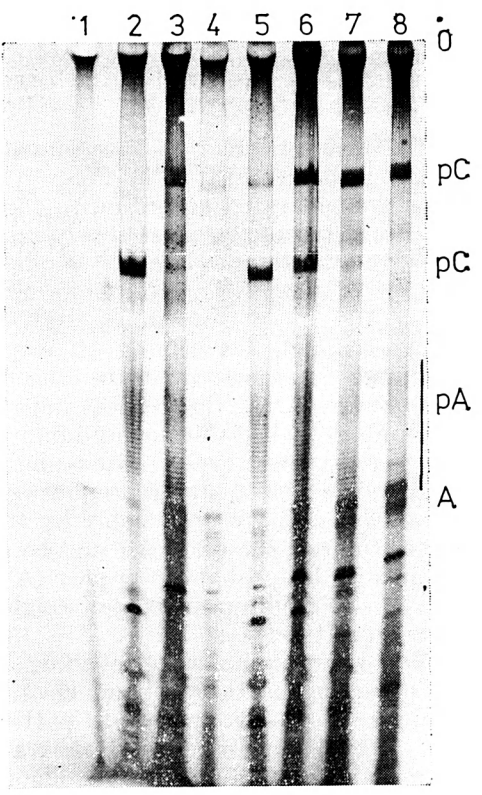


Fig. 1 **Fig. 2**

Saparation of RNase T1 resistant oligonucleotides of FMDV-RNA on a 11.4% polyacrylamide gel at pH 8.3 in 6 mol/l-urea

Detection by silver staining (see Materials and Methods). Direction of electrophoresis is from the top to the bottom.

Fig. 1. Electrophoretic conditions were 165 min at 30 mA at 20 °C; lane 1 and 2 show O_1-oligonucleotides from a density gradient purified vRNA; lane 3, 6, and 9: vRNA of O_1, A_5, C_1 resp. without RNase T1; lane 4 and 5: A_5-oligonucleotides; lane 7 and 8: C_1-oligonucleotides; applied amount was about 45 μg each; pC and pA denote the position of poly (C) and poly (A), respectively. O indicates the origin of the gels. Note the bands marked A, that differ in the positions in type O_1, A_5 and C_1, see also Fig. 2. The vRNA samples were stored as alcohol-precipitated material for 3 years at — 20 °C before T1 digestion.

Fig. 2. Time of electrophoresis: 220 min at 36 mA. The dye marker has migrated 26 cm. The incubation time in $AgNO_3$ was 105 min; lane 1: O_1-oligonucleotides; the applied amount of less than 11 μg was not sufficient; lane 2: C_1-oligonucleotides; the applied amount was about 40 μg; lane 3 and 8: about 50 and 25 μg of A_5-vRNA; lane 5: less than 15 μg A_5- and about 27 μg C_1-vRNA; lane 7: about 25 μg A_5 and about 10 μg C_1-vRNA. The vRNA samples were prepared freshly before T1 digestion.

2 times with water and equilibrated for 2 hr in 1—2 l of 0.011 mol/l $AgNO_3$ (Feinchemie Sebnitz, G.D.R.). After a further short washing the gel was incubated in 0.75 mol/l NaOH, 0.1 mol/l HCHO, 0.0023 mol/l $Na(BH_4)$ for 10—20 min, then after washing for 30 min in 5% acetic acid the bands were photographed (NP 20, ORWO, Wolfen, G.D.R.). In some cases the gel was first stored in 0.07 mol/l Na_2CO_3.

The results with one-dimensional PAGE of oligonucleotides of FMDV-RNA are demonstrated in Figs. 1 and 2. RNase T1 resistant oligonucleotides are separated according to their size. The poly cytidilic acid (poly C) was consisted of the most slowly migrating bands. Under our conditions we were able to detect about 20 bands using about 20— 40 μg vRNA. Concentration of the RNA was determined by UV-absorption measurement. From 100 ml of infectious BHK-cell culture fluid about 40 μg vRNA were obtained. Performing all steps of silver staining in a clear plastic container has proved to be the best procedure. Applying only small amounts of RNA, the gel was kept up for 20 min in NaOH-formaldehyde-sodiumborohydrid solution and immediately photographed. As seen in Fig. 1, the tested viruses type O_1Lausanne, A_5Riems, and C_1Teterow can be clearly distinguished. The difference between the viruses A_5 and C_1 was marked significantly and reproducebly by the size of the poly (C) tract. Differences of positions of the band A could be also seen. The heterogeneous component pA in Fig. 1a, probably the poly (A) tract, is to be seen especially in lanes 7 and 8. The medium molecular weight of poly (A) seems to be higher in type C_1 than in type A_5.

Up to now radioactive labelling and autoradiography have been applied for detecting bands in oligonucleotide mapping. Our results show that one can get good oligonucleotide patterns with relatively small quantities of RNA ($\geqq$25 μg). Whereas Harris and Brown (1976) and (1977) could demonstrate lower quantities of oligonucleotides by P^{32}-labelling than we by silver staining, La Torre *et al.* (1982) found more bands in the formamide gel. The latter authors could already detect the differences between the upper bands of several FMDV-oligonucleotide maps. Our procedure for oligonucleotide mapping of RNA is to be further refined especially in regard of the conditions of electrophoretic separation and silver staining. A great advantage of silver staining is the fact that there is no need of application of radioactive isotopes.

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