Polynucleotide Sequence Homologies among the RNAs of Foot-and-Mouth Disease Virus Types A, C and O

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SUMMARY

The labelled RNAs of the three European types of foot-and-mouth disease virus (A2-sVAIN; C-OBERBAYERN; O1-LOMBARDY) have been cross-hybridized with a large excess of denatured unlabelled foot-and-mouth disease virus-specific double-stranded RNA of the respective virus types. The degree of double-strand reformation in the heterologous reactions was taken as a measure of the extent to which polynucleotide sequences were shared by the virus RNAs. Different degrees of hybridization were obtained across different pairs of virus types: A2-sVAIN with O1-LOMBARDY = 65 %, A2-sVAIN with C-OBERBAYERN = 44 %, O1-LOMBARDY with C-OBERBAYERN = 45 %.

INTRODUCTION

Young, Hoyer & Martin (1968) described polynucleotide sequence homologies among the RNAs of poliovirus types 1, 2 and 3. Recently, Scholtissek & Rott (1969) reported hybridization studies with influenza virus RNAs; these authors used negative strand RNA synthesized in vitro for hybridization with RNA isolated from different myxoviruses. Within the foot-and-mouth disease virus (FMDV) system there are seven serologically and immunologically different types and many subtypes. Until now nothing has been known about common polynucleotide sequences between and within the FMDV types and knowledge of this might give much more information on genetic relationships than is available through serology.

In this paper experiments are presented in which radioactively labelled FMDV-RNA of the three European types was hybridized with double-stranded RNA isolated from BHK cells infected with the respective FMDV types.

METHODS

Viruses and antisera. For virus propagation, FMDV strains adapted to BHK cell tissue culture were used; these strains were A2-sVAIN (A2s), O1-LOMBARDY (O1L) and C-OBERBAYERN (Co). The virus strains were identified by the complement-fixation test as described by Traub & Möhlmann (1943). For exact determination of the FMDV subtypes the technique of Rouminantzeff, Stellmann & Dubouclard (1965) was followed.

Preparation of RNA from purified FMDV. BHK 21 cell monolayer cultures were infected with 10 p.f.u./cell of FMDV and incubated for 3 hr at 37° with VM3A maintenance medium (Schwöbel & Siedentopf, 1961), which contained 20 µC/ml. of [32P]orthophosphate and

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1 µg./ml. actinomycin D. Three hr after infection the virus was concentrated and purified by polyethylene glycol precipitation and ultracentrifugation as described by Kaaden et al. (1971).

Unlabelled virus was produced by infection of BHK 21 cell cultures with < 1 p.f.u./cell and incubation overnight at 37° with Lavit medium (Serva, Heidelberg, Germany). The virus was purified and concentrated as above.

Unlabelled and labelled RNAs were isolated from the purified FMDV using the phenol extraction method of Bachrach (1960). The virus RNA was dissolved in 2 × SSC (0.3 M-NaCl, 0.03 M-sodium citrate). The specific activity of the labelled RNA was 3 × 10⁶ counts/min./µg. Samples containing 0.04 µg. RNA corresponding to 12,000 counts/min. were used for hybridization.

Isolation of double-stranded FMDV-specific RNA. The double-stranded RNA was prepared from FMDV-infected cells as described by Colby & Duesberg (1969). The extracted double-stranded RNA was dissolved in a buffer solution containing 10 mM-tris-HCl, 1 mM-EDTA and 0.15 M-NaCl, pH 7.5. It was subsequently purified by column chromatography on Sepharose 4B (Pharmacia, Uppsala, Sweden) and precipitated overnight with ethanol (67 %, v/v) at −20°.

Denaturation of double-stranded RNA. Essentially the technique published by Nayak & Baluda (1969) was followed. The double-stranded RNA was suspended in 0.01 × SSC, heated in sealed ampoules for 10 min. at 95° and chilled in an ice bath. The denatured double-stranded RNA was adjusted to double SSC concentration and used for the hybridization experiments.

Hybridization experiments. The hybridization procedure used was modified from that of Feix et al. (1967). We could not use the procedure described by Scholtissek & Rott (1969), because the in vitro product synthesized with the help of FMDV-induced polymerase consisted of plus stranded virus RNA (Dietzschold & Ahl, 1970). The labelled FMDV RNA (0.04 µg., 12,000 counts/min.) suspended in 0.2 ml. of 2 × SSC was annealed for 14 hr at 68° in the presence of increasing concentrations of denatured FMDV double-stranded RNA. Each sample was digested with 50 µg. of pancreatic-RNase (Serva, Heidelberg, W. Germany) and 50 units of T1 RNase (Serva, Heidelberg, W. Germany) for 20 min. at 20°. After addition of serum albumin (300 µg.) as carrier protein the non-digested RNA was precipitated with ice-cold 5 % (w/v) trichloroacetic acid containing 0.1 M-pyrophosphate. The precipitates were collected on membrane filters (SM 11305, Satorius, Membranfilter GmbH, Göttingen, W. Germany) and washed five times with trichloroacetic acid. Dried filters were transferred to glass vials containing a toluene scintillator for determination of radioactivity.

For competition experiments a mixture consisting of 0.04 µg. ³²P-labelled virus RNA and an excess of unlabelled FMDV-specific denatured double-stranded RNA (15 µg.) was heated for 5 min. at 95° and annealed for 14 hr at 68° in the presence of increasing amounts of unlabelled virus RNA. The samples were digested with RNase and the radioactivity of the acid-insoluble material was determined as already described.

RESULTS

Cross-hybridization

Labelled FMDV-RNAs of the three European types were used in our hybridization studies. Experiments were performed to determine the quantity of double-stranded RNA required to give maximum re-annealing with labelled virus RNA of the three types. Fig. 1 shows a saturation experiment with denatured A2s double-stranded RNA. A plateau was
Cross-hybridization with FMDV-RNAs

reached for mixtures containing 10 μg. of double-stranded RNA; no further reannealing occurred up to 16 μg. of double-stranded RNA contained in the mixture. Furthermore, different degrees of hybridization occurred with the three labelled RNAs used. The highest degree was reached with the homologous [32P] A2s RNA; the percentage of hybridization with heterologous RNAs was clearly below this value. Fig. 2 and 3 represent the corresponding saturation experiments with Co double-stranded RNA and OI1 double-stranded RNA, respectively. In all three saturation experiments the values of the homologous hybridization rates exceeded 90%. Table 1 summarizes the data for the three FMDV strains tested. In each case the percentage of hybridizability was calculated as the mean of 15
determinations of the amount of hybridization between [32P] FMDV-RNA (0.04 μg.) and a saturating quantity of unlabelled denatured double-stranded RNA (20 μg.). The deviation from the mean was less than 2% for the FMDV strains tested. The extent of heterologous hybridization was related to that in the homologous reaction by taking this as 100%. When 32P-labelled RNA was isolated from uninfected BHK cells this did not hybridize with the FMDV-specific double-stranded RNA.

Competition

The specificity of the homologous and heterologous hybridization reactions was demonstrated in experiments on competition: Fig. 4 illustrates the results of an isotope specific dilution assay. When the hybridization of 20 μg. A2s double-stranded RNA and 0.04 μg. determinations of the amount of hybridization between [32P] FMDV-RNA (0.04 μg.) and a saturating quantity of unlabelled denatured double-stranded RNA (20 μg.). The deviation from the mean was less than 2% for the FMDV strains tested. The extent of heterologous hybridization was related to that in the homologous reaction by taking this as 100%. When 32P-labelled RNA was isolated from uninfected BHK cells this did not hybridize with the FMDV-specific double-stranded RNA.

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Table 1. Hybridization percentage between unlabelled denatured double-stranded RNAs at saturation and labelled parental RNAs of three different types of FMDV

<table>
<thead>
<tr>
<th>Strain of unlabelled denatured double-stranded RNA</th>
<th>Strain of parental [32P] RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hybridization as % of that in homologous reaction</td>
</tr>
<tr>
<td></td>
<td>OIL</td>
</tr>
<tr>
<td>OIL</td>
<td>100</td>
</tr>
<tr>
<td>A2S</td>
<td>63</td>
</tr>
<tr>
<td>Co</td>
<td>46</td>
</tr>
</tbody>
</table>

Homologous hybridization was taken as 100%. A high excess of BHK cell RNA did not hybridize significantly.

Fig. 2. Hybridization of unlabelled denatured OIL double-stranded RNA with 32P-labelled parental RNA from different types of FMDV. • OIL; ○—○, A2S; □—□, Co.

[32P] A2S RNA was made in the presence of increased amounts of unlabelled A2S RNA, the [32P] A2S RNA was diluted out and the RNase-resistant [32P] RNA was reduced to less than 10%. Unlabelled OIL RNA and Co RNA also competed with [32P] A2S RNA but only to about 61 and 42%, respectively; 200 μg. of unlabelled BHK RNA did not compete with the [32P] A2S RNA.

In competition experiments the three virus RNAs showed an inverse reaction when compared with the corresponding saturation experiments.
Cross hybridization with mixtures of two different unlabelled FMDV-specific double-stranded RNAs

By hybridization with a mixture of unlabelled FMDV-specific double-stranded RNAs of two strains at the level of saturation it was possible to determine which parts of the two double-stranded RNAs were involved in hybridization.

Table 2 demonstrates that the polynucleotide sequences of the double-stranded RNA which hybridized with a given ^32P virus RNA overlapped to a large extent.

If there are any additive homologies, as may be indicated by the results obtained in the mixtures of A2s/O1L and Co/O1L, then they are very small.

![Graph](image)

**Fig. 3.** Hybridization of unlabelled denatured Co double-stranded RNA with ^32P-labelled parental RNA from different types of FMDV. □—□, Co; ●—●, O1L; ○—○, A2s.

**Table 2.** Hybridization percentage of ^32P-labelled parental RNAs with mixtures of two different types of unlabelled denatured double-stranded RNA at saturation

<table>
<thead>
<tr>
<th>Strains of unlabelled, denatured double-stranded RNA</th>
<th>Strain of parental [^32P] RNA</th>
<th>Hybridization as % of that in homologous reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2s + Co</td>
<td>O1L</td>
<td>65</td>
</tr>
<tr>
<td>A2s + O1L</td>
<td>Co</td>
<td>51</td>
</tr>
<tr>
<td>Co + O1L</td>
<td>A2s</td>
<td>70</td>
</tr>
</tbody>
</table>

Homologous hybridization was taken as 100%.
DISCUSSION

In this paper significant genetic similarities among the three types of European foot-and-mouth disease viruses are demonstrated. Significant cross-hybridization was found between virus-specific double-stranded RNAs isolated from cells infected with strains A2s, Co or O1L and the $^{32}$P-labelled virus RNAs from these virus strains.

Approximately 45 to 65% of the polynucleotide sequences are shared by the RNAs of the three antigenic types of FMDV. The strain Co, with only about 45% base homology as compared with the strains A2s and O1L, showed the least genetic relatedness. Hybridization experiments with a mixture of saturating amounts of two unlabelled virus-specific double-stranded RNAs indicated that the shared polynucleotide sequences of the three FMDV types overlapped to a large extent. Our results on overlapping are similar to those obtained by Scholtissek & Rott (1969) and Young et al. (1968) for myxoviruses and polioviruses, respectively. The presence of polynucleotide sequence homologies among the three FMDV strains is consistent with the antigenic relationship which operates in cross-immunization (Wittmann, 1966). An antigenic relationship between the FMDV types was also postulated by Brown, Cartwright & Newman (1964), who found that FMDV antibodies precipitate both homotypic and heterotypic virus.

![Graph](image)

Fig. 4. Isotope specific dilution assay of a mixture of $^{32}$P-labelled parental A2s RNA and excess of unlabelled denatured A2s double-stranded RNA in the presence of increased amounts of unlabelled parental RNAs of three different types of FMDV. ○ — ○, A2s; • — •, O1L; □ — □, Co. 200 μg. of unlabelled BHK RNA did not compete with the $[^{32}$P] A2s RNA.
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REFERENCES


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