An Assessment by Competition Hybridization of the Sequence Homology between the RNAs of the Seven Serotypes of FMDV

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(Accepted 4 July 1977)

SUMMARY

A comparison has been made of the RNAs of the seven serotypes of foot-and-mouth disease virus (FMDV) by competition hybridization. Homology among the three European serotypes A, O, C and the Asia I serotype was 60 to 70%. Similar homologies were found among the three Southern African Territories serotypes (SAT 1, SAT 2, SAT 3), but homology between the two groups was much lower (25 to 40%). Homology between the RNAs of subtypes within serotypes A and O was greater than 70%. Double competition experiments with the European serotypes indicate sharing of nucleotide sequences.

INTRODUCTION

There are seven distinct serotypes of foot-and-mouth disease virus (FMDV). These are the European or classical types O, A and C, the Southern African Territories types SAT 1, SAT 2 and SAT 3, and Asia I. Types O, A and C have been found in Europe, South America, Asia and generally in the northern part of Africa though the range is extending. The three SAT types were first detected in Southern Africa but again the range is extending in Africa and SAT 1 has appeared in the Middle East. Asia I occurs in Asian countries from the Eastern Mediterranean to the Far East.

These types were first differentiated by the lack of cross-immunity following infection by distinct types but the development of laboratory tests led to more rapid detection of differences between types and within types (sub-type strains). The occurrence of the different serotypes and the methods used for their recognition were reviewed by Brooksby (1958).

The variation measured by these tests is expressed on the virus capsid proteins which are coded for by the 30 to 40% of the virus RNA at the 5' end (Sangar et al. 1977). It is not known, however, to what extent variation is limited to this region of the genome. In experiments to study differences between the RNAs of different isolates of FMDV, Dietzschold et al. (1971) demonstrated that the sequence homology of the RNAs of examples of each of the European types of the virus was between 44 and 65%. A study of the RNA of some of the type O subtypes showed homology of between 50 and 70% (Dietzschold, Kaaden & Ahl, 1972), but an unexpectedly low level of homology (48%) was detected between some of the subtypes.

As part of a programme to examine antigenic variation in FMDV, we are attempting to correlate variation with differences in the RNA. In this paper the sequence relationships between the RNAs of all seven serotypes are examined in more detail.
METHODS

Viruses. The following strains adapted to BHK 21 cell tissue culture were used: A5 (Westerwald), A10 (Kemron), A24 (Iraq 24/64), A24 (Cruziero), O1 (BFS I848), O1 (Ovl), C2 (C97), SAT 1/2 (RV II/37), SAT 2/1 (RHO I/48), SAT 3/1 (RV 7/34) and Asia 1/1 (Pak 1/54). These were obtained from the World Reference Centre for FMD, at this Institute.

Preparation and purification of radioactively labelled and unlabelled virus. BHK cell monolayers containing approx. 10^9 cells were infected at 37 °C with virus at a multiplicity of about 20. After 30 min to allow adsorption, the cells were washed with Eagle's medium, 20 ml of fresh medium added and the cells incubated at 37 °C. Radioactive label (300 μCi 5-3H-uridine, 26 Ci/mmol, Radiochemical Centre, Amersham) in 20 ml Eagle's medium was added to the infected cells 1 to 1.5 h after infection and the virus was purified from the medium at the end of the growth cycle as described previously (Harris & Brown, 1977). Unlabelled virus was prepared from the medium from 4 × 10^9 BHK cells infected at low multiplicity; after concentration by precipitation with 50% (NH₄)₂SO₄, buffered to pH 7.6 with 0.04 M phosphate buffer, it was purified as for radioactive virus.

Extraction and purification of RNA from virus particles. The sucrose gradient fractions containing virus were diluted at least threefold with 0.15 M-NaCl, 0.05 M-tris-HCl, 0.005 M-EDTA, pH 7.6 (TNE buffer) containing 0.1% SDS, 0.01 #g of E. coli tRNA added, extracted twice with a 1:1 mixture of phenol:chloroform and the RNA in the aqueous phase precipitated with 2 vol. ethanol overnight at -20 °C. The virus RNA was always purified further by sucrose gradient centrifugation. The RNA was dissolved in 0.5 ml 0.1 M-acetate, 0.1% SDS, pH 5.0, layered on to a 14 ml 5 to 25% (w/v) sucrose gradient containing 0.1 M-acetate/0.1% SDS, pH 5.0, and centrifuged at 60000 g for 16 h at 20 °C (Beckman SW 27.1 rotor). The fractions comprising the RNA peak (at about 37S) were pooled, 100 μg of carrier E. coli RNA added and the RNA precipitated with 2 vol. of ethanol overnight at -20 °C. Unlabelled virus RNA was extracted and purified in a similar way, but without the tRNA carrier.

Extraction of double-stranded RNA from infected cells. Double-stranded RNA (ds RNA) was extracted and purified from about 4 × 10^9 virus infected cells by phenol extraction and 2 M-LiCl precipitation. The ds RNA in the LiCl soluble fraction was purified further from low mol. wt. single-stranded RNA (ss RNA) by centrifuging on 14 ml 5 to 25% (w/v) sucrose gradients in 0.1 M-acetate/0.1% SDS, pH 5.0, for 16 h at 80000 g (Beckman SW 27.1 rotor) at 20 °C. The ds RNA was recovered from the gradient by ethanol precipitation. These procedures have been described in detail previously (Harris & Brown, 1977).

Molecular hybridization. Saturation and competition hybridization experiments were done in duplicate in 0.2 ml of 50% formamide, 5 × SSC at 50 °C exactly as described by Harris & Brown (1977). The competitor RNAs were dissolved in 50% formamide, 5 × SSC to give a concentration of 10 μg/ml. The ds RNA concentration was adjusted so that at zero competitor concentration there were between 1000 and 2000 ct/min in the hybrid as determined by saturation hybridization (Harris & Brown, 1977). In double competition experiments, the two competing unlabelled RNAs were added in equal proportions to the ds RNA/3H-ss RNA mixture. The results obtained with the hybridization procedures were handled as described in detail by Darby & Minson (1973). Briefly, the reciprocal plots of the competition hybridizations give intercepts on the 1/f axis, which allow the determination of f, the fraction of RNA displaced by the competitor at infinite competitor RNA concentration.

The values given in Tables 1 to 3 are the mean values for the 1/f intercepts from two or
Competition hybridization of FMDV RNAs

more experiments; the regression coefficient, \( r_{xy} \), for each of these reciprocal plots (see Fig. 1) was \(< 0.95\). The same results were obtained when the graphical procedure of Eisenenthal & Cornish-Bowden (1974) was used directly to ascertain the value of \( f \).

RESULTS

Competition hybridization of FMDV serotypes

RNA extracted from FMDV, type A\(_{10}\) labelled with \(^3\)H-uridine was annealed to denatured double-stranded RNA, extracted from BHK cells infected with the same virus, in the presence of competing unlabelled RNAs from the homologous virus and from two other European serotypes (types O and C) and the Asia I serotype. Fig. 1(a) shows that the reciprocal plot of the data obtained for the homologous competition crosses the \( 1/f \) axis at 1.0, as expected. However, the heterologous competition crosses the \( 1/f \) axis at 1.5, indicating a homology of competing RNAs with the A\(_{10}\) RNA of 64 to 67\%. This degree of homology is higher than that obtained with RNA from other isolates of types O, A and C using saturation hybridization (Dietzschold et al. 1970).

A similar experiment using unlabelled ds RNA from SAT I virus infected cells and \(^3\)H-uridine labelled SAT I virus ss RNA, in competition with the homologous RNA and with RNA from the other SAT serotypes is shown in Fig. 1(b). The result for the homologous competition was again 100\% whereas for the heterologous competitions values of 65 to 68\% were obtained.

Converse experiments in which each of these competitions was done with labelled virus RNA and unlabelled ds RNA of types O, C, Asia I, SAT 2 and SAT 3 are shown in Table 1. The same sequence homologies were obtained within the two groups of viruses irrespective of the virus used as source of radioactive RNA. Table 1 also shows that a similar level of sequence homology exists within the three SAT serotypes as within the three European and Asia I serotypes.

The sequence homology between the European and Asia I serotypes on the one hand and the SAT serotypes on the other was also assessed using the same methods (Table 1). Much lower levels of homology were found between the SAT and European-Asia serotypes (25 to 40\%) than within the two groups (60 to 70\%).

Double competition experiments

Double competition experiments in which a mixture of two heterologous ss RNAs are allowed to compete with the homologous labelled ss RNA for complementary RNA were used to determine whether the homologous sequences in the European serotypes were shared. The results in Table 2 suggest that there is some small but significant additional competition when compared to either of the competing RNAs alone; this is in agreement with the results obtained by Dietzschold et al. (1971) using saturation hybridization.

Competition hybridization of FMDV subtypes within the A and O serotypes

In a comparison of the sequence homology of the RNAs of subtypes of type O FMDV by saturation hybridization, Dietzschold et al. (1972) found considerable variation in the homologies ranging from 48 to 73\%. The results that we have obtained with some of the subtypes of type A FMDV when compared by competition hybridization using labelled A\(_{10}\) virus RNA and ds RNA from A\(_{10}\) infected cells are shown in Table 3. The levels of...
Fig. 1. Reciprocal plots of competition hybridization experiments: (a) $^3$H-uridine-A$_{10}$ ss RNA annealed to A$_{10}$ ds RNA in the presence of increasing amounts of unlabelled A$_{10}$ ss RNA (○—○), C$_2$ ss RNA (▲—▲), O$_6$ ss RNA (○—○) or Asia 1/1 ss RNA (■—■); (b) $^3$H-uridine-SAT 1/2 ss RNA annealed to SAT 1/2 ds RNA in the presence of increasing amounts of unlabelled SAT 1/2 ss RNA (○—○), SAT 2/1 ss RNA (■—■) or SAT 3/1 ss RNA (▲—▲).

Table 1. Sequence homology of the RNAs of the seven serotypes of FMDV

<table>
<thead>
<tr>
<th>Unlabelled ss RNA competitor</th>
<th>A$_{10}$</th>
<th>O$_6$</th>
<th>C$_2$</th>
<th>Asia 1/1</th>
<th>SAT 1/2</th>
<th>SAT 2/1</th>
<th>SAT 3/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A$_{10}$</td>
<td>100</td>
<td>66</td>
<td>67</td>
<td>65</td>
<td>20</td>
<td>—</td>
<td>33</td>
</tr>
<tr>
<td>O$_6$</td>
<td>67</td>
<td>100</td>
<td>68</td>
<td>62</td>
<td>26</td>
<td>—</td>
<td>33</td>
</tr>
<tr>
<td>C$_2$</td>
<td>67</td>
<td>70</td>
<td>100</td>
<td>57</td>
<td>37</td>
<td>—</td>
<td>37</td>
</tr>
<tr>
<td>Asia 1/1</td>
<td>64</td>
<td>64</td>
<td>60</td>
<td>100</td>
<td>26</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>SAT 1/2</td>
<td>25</td>
<td>25</td>
<td>28</td>
<td>—</td>
<td>100</td>
<td>64</td>
<td>68</td>
</tr>
<tr>
<td>SAT 2/1</td>
<td>37</td>
<td>40</td>
<td>28</td>
<td>—</td>
<td>65</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td>SAT 3/1</td>
<td>37</td>
<td>27</td>
<td>—</td>
<td>—</td>
<td>68</td>
<td>62</td>
<td>100</td>
</tr>
</tbody>
</table>

The sequence homology found were consistently above 70%, showing that there is more sequence homology between subtypes within serotype A than between serotypes. The two type O subtypes that were compared to each other gave a similar level of homology (Table 3).

**DISCUSSION**

Sequence homologies of 64 to 68% were found between the RNAs of FMDV types A, O and C (Table 1) when assessed in an accurate competition hybridization assay (see Methods). This level of homology is higher than that obtained previously by Dietzschold et al. (1971) using saturation hybridization under different reaction conditions. As the degree of nucleotide mismatch in the hybrids that is tolerated has not been examined, it is possible that the difference in values is due to methodology. It is also possible that the
subtypes used by Dietzschold et al. (1971) as representative of serotypes A, O and C have lower homologies. We consider this latter alternative to be unlikely as the subtypes we studied have RNA homologies above 70% (Table 3); moreover, the serotype annealing results (Table 1) are internally consistent (cf. Dietzschold et al. 1971).

The European and Asia I serotypes clearly form a group distinct from the SAT serotypes. The homology between these two groups (25 to 40%, Table 1) is lower than that within groups (60 to 70%) and raises interesting questions concerning their evolution. The results in Table 3 suggest that there is more sequence homology between subtypes of serotype A than there is between serotypes. Although the subtypes we examined are clearly different by complement fixation tests, the homology between subtypes and serotypes is similar to that found between the three serotypes of poliovirus and two strains of poliovirus, type I (Young, Hoyer & Martin, 1968). Dietzschold et al. (1972), however, found that subtypes of serotype O were as distinct in RNA homology tests as different serotypes. The discrepancy between our results and those of Dietzschold et al. (1972) cannot be resolved fully until more subtypes have been examined. Nevertheless, the homology with the O subtypes, which were isolated at an interval of forty years (80%, Table 3) is similar to that between the A subtypes.

It is difficult to relate sequence homology, which is a measure of variation in the total RNA, to antigenic variation, which is a reflection of difference in a maximum of 35 to 40% of the RNA (that coding for the structural polypeptides). Further detailed analysis of the biochemistry of subtypes of FMDV, relating RNA structure to polypeptide composition and antigenicity, should provide some insight into antigenic variation in this virus.

We wish to thank Dr J. R. Crowther for helpful discussion. K. J. H. R. gratefully acknowledges the award of a Wellcome Foundation Studentship.
REFERENCES


(Received 3 June 1977)