Size Differences in the Ribonucleic Acids of Feline Leukaemia Viruses

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SUMMARY

Electrophoretic analysis of native and denatured RNA of feline leukaemia virus (FeLV) has revealed small but significant size differences between the RNAs of a number of FeLV isolates. The mol. wt. of the denatured or subunit RNA has been estimated to range from 2.2 to 2.6 x 10^6 in the different isolates, each of which belonged to one or more FeLV subgroups (A, B and C). The RNA of subgroup A virus was smaller than that of subgroup B virus, while the RNA of subgroup C virus appeared to be intermediate in size between the RNAs of A and B subgroup viruses. Size differences were, however, also found between RNAs of FeLV isolates of a single subgroup (A), indicating that no simple relationship exists between RNA size and virus subgroup.

INTRODUCTION

Feline leukaemia viruses (FeLV) have been classified in three subgroups by their specific interference patterns (Sarma & Log, 1971). In addition, viruses of each subgroup differ in their surface antigens (Sarma & Log, 1973) and host range (Jarrett, Laird & Hay, 1973). The present studies attempt to establish whether these differences are associated with detectable differences in the size of the virus genome.

Studies on avian tumour viruses by Duesberg & Vogt (1970) indicated that the ability of a virus to transform chick cells was related to the presence of an RNA subunit which was lacking in non-transforming variants. Spontaneous loss of this subunit from a transforming virus was associated with conversion to a non-transforming variant (Martin & Duesberg, 1972). Size differences in the high mol. wt. RNAs of certain avian virus isolates were found by Bolognesi & Graf (1971) not to be related to their biological or antigenic properties, but mutagenization of a non-transforming sarcoma virus was also accompanied by a loss of RNA.

In common with other RNA tumour viruses the native (75 S) RNA of FeLV has a mol. wt. of about 11 x 10^6; disassociation to subunits of about 2.5 x 10^6 occurs on denaturation by heat or chemicals (Jarrett et al. 1971; Whalley, 1973). This report describes the use of composite gels of acrylamide and agarose (Peacock & Dingman, 1968) to compare the sizes of RNAs from several FeLV isolates of different subgroups. Estimates have also been made of the mol. wt. of the RNA subunits from these isolates and a possible relationship to their biological properties is discussed.
METHODS

Virus isolates. The isolates of FeLV used were FeLV-I and FeLV-5 from this laboratory (Jarrett, Laird & Hay, 1972) and FeLV-A, B and C which were a gift from Dr Padman Sarma.

Virus purification and RNA extraction. The preparation from feline embryo tissue culture cells of FeLV labelled with [3H]-uridine or [32p] has been described previously (Jarrett et al. 1971). The purified virus was treated with 1% Sarkosyl-NL97 (Geigy Ltd.) and 250 μg/ml pronase (Calbiochem Ltd.) for 15 min at 37 °C. The extracted RNA was then sedimented through a gradient of 5 to 20% sucrose, containing 0.5% Sarkosyl in TS buffer (0.01 M-tris, 0.1 M-sodium chloride and 0.001 M-EDTA, pH 7.5), for 35 min at 60000 rev/min in a Beckman L2-65B ultracentrifuge. The gradient fractions containing the native 75 S RNA were pooled along with 50 μg of highly polymerized yeast RNA (BDH Chemicals Ltd.), and stored at −20 °C in 67% ethanol. Prior to electrophoresis, the RNA was centrifuged at 3000 rev/min for 30 min and the pellet was re-suspended in a 1:4 dilution of the electrode buffer described below. When comparing RNAs from two virus subgroups, differentially labelled ([32p] and [3H]) RNAs were pooled in the 67% ethanol suspension, before spinning down for electrophoresis.

RNA markers. Radioactively labelled EMC virus was prepared from infected BHK-21/C13 cells grown in Eagle’s medium containing 10% foetal bovine serum and 10 μCi [3H]-uridine/ml (The Radiochemical Centre, Amersham). The EMC virus was purified by equilibrium density-gradient centrifuging in caesium chloride of mean density 1.33 g/ml. The RNA was extracted from the banded virus by Sarkosyl/pronase treatment and was sedimented through a 5 to 20% sucrose gradient. The peak fractions were pooled and stored in 67% ethanol at −20 °C.

Radioactively labelled ribosomal RNA was prepared from feline embryo cells grown in medium containing 5 μCi [3H]-uridine/ml. Ribosomes were isolated from the cytoplasmic extract as described by Penman, Greenberg & Willems (1969), and RNA was purified by phenol extraction followed by sucrose gradient centrifuging.

Polyacrylamide-agarose gel electrophoresis. The procedure used was based on that of Peacock & Dingman (1968) and developed in collaboration with Dr R. Mayol at the California Institute of Technology. Cylindrical gels containing 1.5% acrylamide and 0.5% agarose were prepared from the following stock solutions: (a) 10% acrylamide (Eastman) (10 g acrylamide and 0.5 g N,N-methylene bis-acrylamide in 100 ml of water), (b) 0.28% ammonium persulphate, (c) 2% agarose (Seakem, Bausch and Lomb) in water, (d) the buffer as described by Loening (1967), was a 0.4 M-tris, 0.5 M-sodium acetate, and 0.02 M-dissodium EDTA, pH 7.8. This buffer was used in preparation of the gels and was diluted fivefold when used in the electrodes. Acrylamide buffer solution was prepared by mixing 1:2 ml of solution (a) 1.6 ml of the undiluted buffer (d) 1.2 ml of water, 10 μl of Sarkosyl, and 5 μl of N,N,N,N-tetramethylene diamine (Koch-Light). Each 6 cm gel was prepared in siliconized glass tubes from 1.0 ml of acrylamide buffer solution, 0.5 ml of solution (b) and 0.5 ml of solution (c). When the gel had fully polymerized (1 h at 25 °C) the lower end of the tube was sealed with dialysis tubing and the upper surface was rinsed with electrode buffer. Prior to the electrophoresis of RNA samples the newly prepared gel was pre-run at a current of 2 to 5 mA for 1 h.

The virus RNA samples were suspended in a 1:4 dilution of the electrode buffer containing 10% sucrose and were layered carefully on top of the gels. A low current (1 to 2 mA) was applied to each gel for 15 min prior to increasing the current to that described in the
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**Fig. 1.** Electrophoresis of native and denatured FeLV RNA. [\(^{32}P\)]-labelled (○–○) FeLV 75 S and 4 S RNAs were mixed in electrophoresis buffer (Loening, 1967) with [\(^{3}H\)]-labelled (●–●) FeLV-1 75 S RNA which had been heated for 2 min at 80 °C. The RNAs were co-electrophoresed in a 1·5 % acrylamide-0·5 % agarose gel for 5 h at 2 mA per gel, at room temperature.

**Fig. legends.** Before use, the gel tubes and the electrode wells were soaked overnight in distilled water containing 0·5 % Sarkosyl and 0·1 % diethyl-pyrocarbonate in an attempt to reduce nuclease activity. After electrophoresis, the gels were extruded from the tubes and were frozen before slicing into 1 mm fractions on a Mickle gel slicer. These fractions were then solubilized overnight in a toluene-based scintillation fluid containing NCS solubilizer (Amersham/Searle), and were analysed on an Intertechnique liquid scintillation counter.

**RESULTS**

*Effect of denaturing FeLV RNA*

Conversion of the native (75 S) FeLV RNA to its subunit form was achieved by heating the RNA in a 1:4 dilution of electrode buffer to 80 °C for 2 min, then chilling in ice. The effect of this treatment is shown in Fig. 1 which shows [\(^{3}H\)]-labelled subunit RNA co-electrophoresed with [\(^{32}P\)]-labelled 75 S and 4 S RNAs from purified virus. The denatured RNA had migrated approximately twice the distance of the native RNA material, supporting the results obtained by sedimentation analysis of FeLV RNA (Jarrett et al. 1971) in which 75 S RNA was changed by denaturation to material with a leading edge of about 37 S. This denatured or subunit RNA characteristically appears to have variable amounts of material migrating ahead of the main peak. This material probably consists of non-specific fragments arising from nuclease or other degradative action on the RNA prior to denaturation and has been observed in similar RNAs of avian and murine RNA tumour viruses (Watson, 1971). Part of this material may, however, be the small (<10 S) component which
appears to be necessary for complete template activity of the virus RNA-dependent DNA polymerase (Canaani & Duesberg, 1972). Under these conditions virus 4 S RNA migrated to the end of the gel.

Comparison of subunit RNA from FeLV of different subgroups

The co-electrophoresis of subunit RNAs from FeLV isolates of different subgroups is shown in Fig. 2. In this series of experiments [¹H]-FeLV-1 (A-subgroup) RNA was compared with [³²P]-labelled RNAs of FeLV of A, B and C subgroups and FeLV-5 (a mixture of A and B subgroups). From Fig. 2(a) it can be seen that the FeLV-1 RNA migrated slightly more rapidly than FeLV-B RNA. This difference was consistently observed and the same result was obtained when the two RNAs were labelled with the reverse isotope, i.e. [³²P]-FeLV-B versus [¹H]-FeLV-1. In a further control experiment the peaks of [¹H]-FeLV-1 RNA and [³²P]-FeLV-1 RNA were exactly co-incident when analysed in a similar gel. The main peak of FeLV-C subunit RNA migrated at the same rate as that of FeLV-1 although there was a shoulder of slightly more slowly moving material (Fig. 2(b)). The RNA of the FeLV-A migrated distinctly more rapidly than that of FeLV-1 although both viruses are of the A subgroup (Fig. 2(c)). The profile of the FeLV-A RNA indicated the presence of at least two and possibly three components which could be a reflexion of mixtures of FeLV existing within the subgroup. This would not be unexpected, since although this FeLV isolate was obtained by end-dilution methods, it has not been grown from cloned virus.
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Fig. 3. Gel electrophoresis of subunit FeLV RNA with marker RNAs. (a) \(^{3}P\)-labelled (○—○) EMC virus RNA was mixed with denatured \(^{3}H\)-labelled (−−−) FeLV-I RNA followed by co-electrophoresis in a 1.5% acrylamide-0.5% agarose gel for 5 h at 2 mA. (b) \(^{3}H\)-labelled (○−○) ribosomal RNA from feline embryo tissue culture cells mixed with denatured \(^{3}P\)-labelled (○−○) FeLV-I RNA followed by co-electrophoresis in a 1.5% acrylamide-0.5% agarose gel for 3 h at 3 mA.

Table 1. Estimated mol. wts. of FeLV RNA subunits*

<table>
<thead>
<tr>
<th>FeLV isolate</th>
<th>Subgroup</th>
<th>Range</th>
<th>Mean</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeLV-A</td>
<td>A</td>
<td>2.09 to 2.24</td>
<td>2.18</td>
<td>±0.01</td>
</tr>
<tr>
<td>FeLV-I</td>
<td>A</td>
<td>2.40 to 2.60</td>
<td>2.51</td>
<td>±0.04</td>
</tr>
<tr>
<td>FeLV-5</td>
<td>AB</td>
<td>2.45 to 2.64</td>
<td>2.55</td>
<td>±0.04</td>
</tr>
<tr>
<td>FeLV-B</td>
<td>B</td>
<td>2.43 to 2.61</td>
<td>2.53</td>
<td>±0.04</td>
</tr>
<tr>
<td>FeLV-C</td>
<td>C</td>
<td>2.40 to 2.60</td>
<td>2.51</td>
<td>±0.04</td>
</tr>
</tbody>
</table>

* Calculated from logarithmic relationship of distance migrated to mol. wt., based on EMC virus RNA and 28 S ribosomal RNA markers (Bishop et al. 1967). The values shown are the results from four separate gel analyses of each type of RNA.

Only a slight difference was observed between the subunit RNAs of FeLV-1 and FeLV-5, the latter appearing to move more slowly.

Although sequence differences in the RNA molecules could also result in altered conformation and hence electrophoretic mobility preliminary analyses indicated that each virus isolate has a similar base composition (unpublished observation). It seems likely therefore that the differences in mobility are due mainly to differences in molecular size.

*Estimation of subunit RNA mol. wt.*

In order to obtain an estimate of the mol. wts. of the subunit RNAs and of the approximate size differences between RNAs of different virus subgroups, EMC virus RNA and ribosomal RNA were co-electrophoresed with FeLV subunit RNA. Fig. 3 shows that the EMC RNA migrated just behind the FeLV subunit RNA while the 28 S ribosomal RNA migrated considerably faster. Assuming a mol. wt. of 2.7 × 10^6 for EMC RNA (Burness, 1970) and 1.8 × 10^6 for 28 S RNA (Loening, 1969) and using a logarithmic relationship of mol. wt. to distance migrated (Bishop, Claybrook & Spiegelman, 1967) the sizes of the different FeLV subunit RNAs have been estimated (Table 1). The mol. wts. range from 2.6 × 10^6 for FeLV-B to 2.2 × 10^6 for the FeLV-A isolate.
Fig. 4. Electrophoresis of native (75 S) FeLV RNAs in 1.5% acrylamide-0.5% agarose gels. [3H]-labelled (●–-●) FeLV-1 RNA was mixed with [32P]-labelled (O--○) RNAs of a number of FeLV isolates of differing antigenic subgroups, and co-electrophoresed as follows: (a) RNAs of [3H]-FeLV-t and [32P]-FeLV-B for 3 h at 5 mA. (b) RNAs of [3H]-FeLV-1 and [32P]-FeLV-C for 3 h at 5 mA. (c) RNAs of [3H]-FeLV-1 and a [32P]-FeLV-A isolate for 17 h at 1.5 mA. (d) RNAs of [3H]-FeLV-1 and [32P]-FeLV-5 for 3.5 h at 5 mA.

Comparison of 75 S RNAs

In view of these differences observed between the subunit RNAs of the various FeLV isolates, the native (75 S) RNAs were also compared (Fig. 4). In general it was found that the pattern was the same as the subunit RNAs, although the differences were less marked. However, when the RNAs were electrophoresed close to the end of the gel as in Fig. 4(c), a more distinct separation was observed. It is possible that the three-dimensional structure of the native leukaemia virus RNA is less affected by small changes in RNA length than the denatured form. The occurrence of variable amounts of more slowly migrating material behind the main peaks was probably due to aggregation after ethanol precipitation. Such aggregation may be removed by heating at 37 °C in 0.01 M-EDTA for 30 min (Bishop et al. 1970).

DISCUSSION

The use of dilute acrylamide and agarose gels has revealed size differences among the RNAs of FeLV isolates from different subgroups. Although such variations have been shown among certain avian RNA tumour viruses (Duesberg & Vogt, 1970; Bolognesi & Graf, 1971) they have not previously been reported for mammalian RNA tumour viruses. The disaggregation upon heat denaturation of the FeLV 75 S RNA into smaller subunits is a feature common to all RNA tumour viruses. The importance of this subunit RNA has been emphasized by the recent pulse-labelling experiments of Cheung et al. (1972) and of
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Canaani, Helm & Duesberg (1973) which indicate that the subunit RNA may in fact be the main intracellular virus RNA synthesized during Rous sarcoma virus replication, subsequently associating within the virus particle to form the 60 to 70 S RNA.

The estimated mol. wts. of FeLV subunit RNA of 2·2 to 2·6 × 10⁶ are similar to that obtained for Rous sarcoma virus by Montagnier, Golde & Vigier (1969), who showed that the RNA subunit sedimented close to EMC virus RNA in both aqueous and dimethyl sulphoxide gradients. The values obtained by Maruyama, Hatanaka & Gilden (1971) from tritiated end group analysis also were in this range (2·2 to 2·9 × 10⁶ for several different species).

The size difference of 1 × 10⁶ to 4 × 10⁶ between subunit RNAs of various FeLV isolates could mean the difference in ability to code for 1 to 3 proteins, possibly envelope glycoproteins, as suggested by the report of Lai & Duesberg (1972) in studies on avian sarcoma and leukosis viruses. It is of considerable interest that the A subgroup FeLV isolates examined have smaller RNAs than the other subgroups and that it is the A subgroup which has a restricted host range (Jarrett et al. 1972). This apparent correlation between the RNA size and ability to grow in human or canine cells may be merely coincidental, and many more isolates would require testing before concluding that such a relationship exists. The observed RNA size differences within FeLV isolates of subgroup A is also evidence that the virus envelope antigens and host range do not have a simple relationship to the RNA content.

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REFERENCES


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