

VIROID-LIKE RNA ENCAPSIDATED IN LUCERNE TRANSIENT STREAK VIRUS

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1. Introduction

Lucerne transient streak virus (LTSV) has been isolated from lucerne crops in Australia and New Zealand [1,2]. The virus was characterised and shown to share many properties with members of the southern bean mosaic virus group [3]. Velvet tobacco mottle virus (VTMoV), solanum nodiflorum mottle virus (SNMV) and subterranean clover mottle virus (SCMoV) have properties similar to those of southern bean mosaic virus (SBMV), but have an unusual RNA complement [4,5]. In addition to virus-like linear single-stranded (ss)-RNA molecules, viroid-like circular ss-RNA molecules were also present [4–6]. Virus- and viroid-like RNA molecules of VTMoV and SNMV have no base sequence homology and both are essential for infection [7,8]. Here we examine the RNA complement of highly purified preparations of LTSV and show it to consist of both virus- and viroid-like RNA molecules.

2. Materials and methods

Isolates of LTSV (AUS [1] and NZ [2]) were maintained and purified from *Chenopodium quinoa* Willd. and *Nicotiana clevelandii* L., respectively. The viruses were purified by extraction in 0.07 M phosphate buffer (pH 7.0) containing 0.1% thioglycolic acid, clarification with a chloroform–carbon tetrachloride mixture and 2–3 cycles of differential centrifugation followed by sucrose density-gradient centrifugation [4]. RNA was isolated from virus preparations by phenol–SDS extraction and analysed by electrophoresis in polyacrylamide gels under denaturing conditions [7]. For electron microscopy in a JEM 100 CX

instrument, virus preparations were stained with 2% uranyl acetate and RNA preparations were spread under denaturing conditions and rotary shadowed [4]. Preparations of RNA 2 and RNA 3 were isolated by polyacrylamide gel electrophoresis under denaturing conditions followed by sucrose density-gradient centrifugation [7]. Thermal denaturation of the RNAs in 1 × SSC buffer (0.15 M NaCl + 0.015 M sodium citrate pH 7.0) was determined as in [7].

3. Results

Preparations of both isolates of LTSV sedimented at the same rate as a single component which contained identical, small isometric particles, 30 nm diam. indistinguishable from those of VTMoV and SBMV [4]. When electrophoresed in polyacrylamide gels under denaturing conditions, LTSV-RNA separated into three main bands, 1, 2 and 3 (fig.1a), like the RNAs from VTMoV (fig.1b) and SNMV (fig.1c). However, RNAs 2 and 3 of LTSV migrated at a significantly faster rate than the corresponding RNA fractions of VTMoV and SNMV (fig.1). The RNA of SBMV migrated as a single major band similar to RNAs 1 of LTSV, VTMoV and SNMV. RNAs from the AUS and NZ isolates of LTSV had indistinguishable RNA components and when coelectrophoresed, RNAs 1, 2 and 3 of each migrated together. Under non-denaturing conditions LTSV-RNA 2 and RNA 3 migrated as a single homogeneous zone. In this respect the RNAs behave similarly to those of VTMoV [4].

RNAs 2 and 3 of both VTMoV and SNMV correspond to circular and linear forms, respectively, of the same RNA species, the linear molecules migrating ahead of the circular ones [6,7]. When preparations of LTSV-RNA were spread under denaturing conditions for electron microscopy, circular molecules

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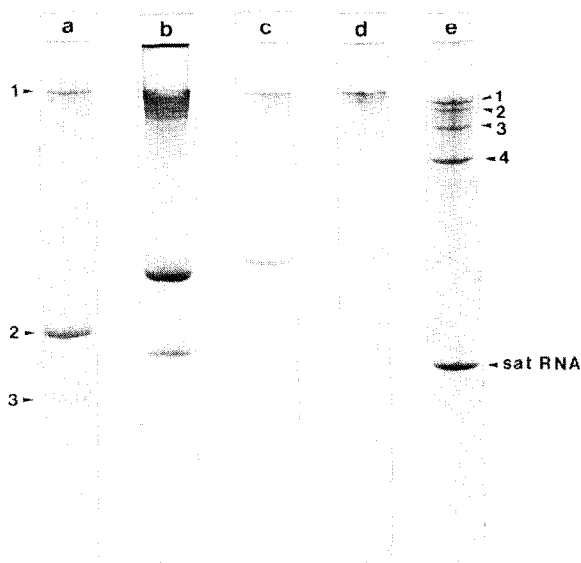


Fig.1. Polyacrylamide-gel electrophoresis of RNA preparations from LTSV-AUS (a), VTMoV (b), SNMV (c), SBMV (d) and CMV containing satellite RNA (e). The RNAs isolated from purified virus preparations by phenol-SDS extraction were subjected to electrophoresis under denaturing conditions [7]; (1-3) on the left refer to the three main bands of RNA; (1-4) and sat-RNA on the right refer to CMV RNAs of relative molecular mass 1.35, 1.16, 0.85, 0.35 and 0.12×10^6 , respectively [9,11].

were observed (fig.2) indicating that the RNA complement of this virus is similar to those of VTMoV and SNMV. The thermal denaturation of RNA 2 produced an absorbance-temperature profile with a sharp transition (fig.3) and a T_m of 70°C . The absorbance temperature profile of RNA 3 is broader than that of RNA 2 with a lower T_m (fig.3). These data indicate that RNA 2 and 3 are viroid-like and that RNA 2 is circular whereas RNA 3 is linear [6,7].

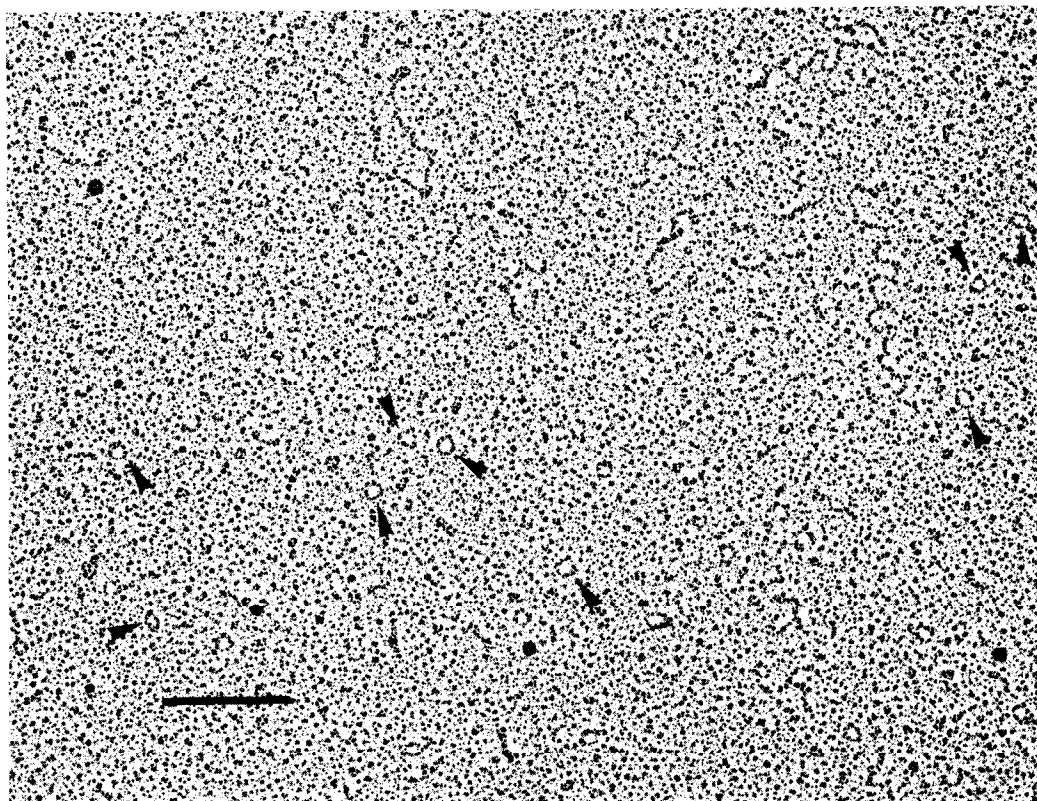


Fig.2. Electron microscopy of RNA isolated from purified preparations of LTSV-AUS. Small circular RNA molecules are indicated by arrowheads. (Bar represents 300 nm.)

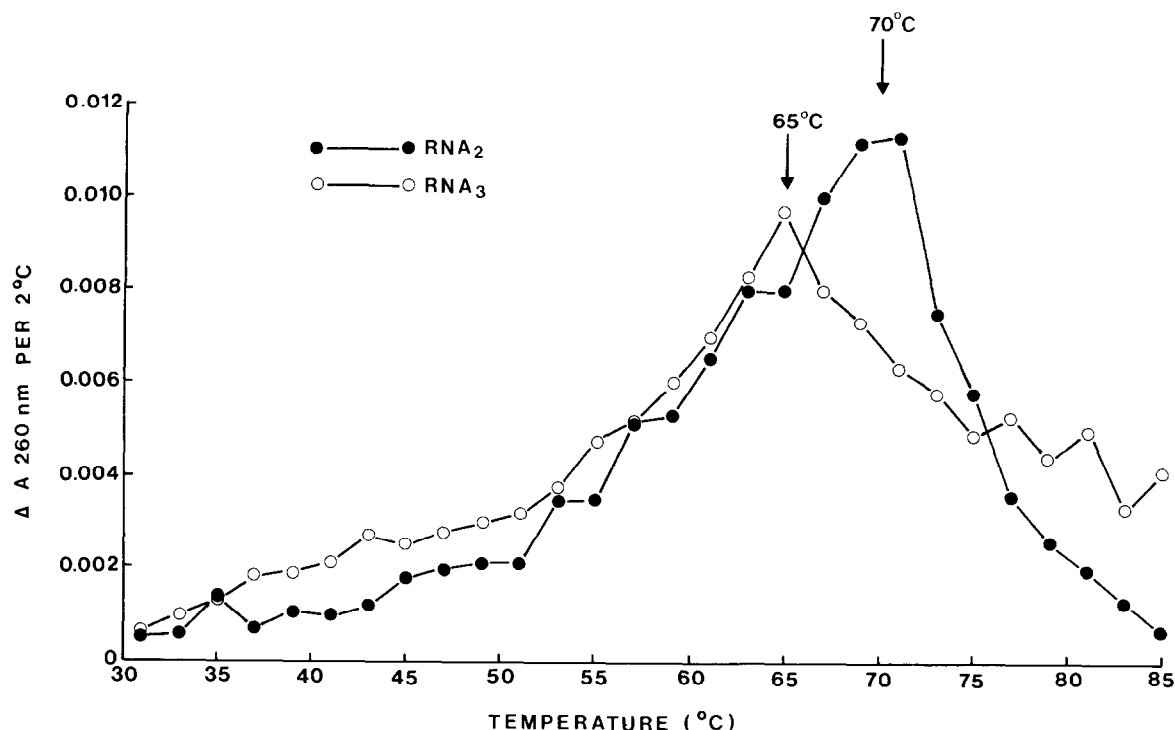


Fig.3. Thermal denaturation profiles of LTSV-RNA 2 and RNA 3 in $1 \times$ SSC buffer. The temperature was increased at $0.5^\circ\text{C}/\text{min}$. The T_m -values are indicated by arrows.

RNA sequence analyses of RNAs 2 of SNMV and VTMoV have established that they consist of 377 and 366 nucleotides, respectively [10]. Similarly, it has been determined that the satellite RNA of cucumber mosaic virus (CMV) [11] is a linear single-stranded molecule 337 nucleotides long [10]. Hence, from comparisons of the electrophoretic mobilities under denaturing conditions of the RNAs 3 of SNMV and VTMoV and the CMV satellite RNA, it was estimated that LTSV-RNA 3 and hence also LTSV-RNA 2 consist of ~ 300 nucleotides.

4. Discussion

It has been suggested that LTSV should be included in the southern bean mosaic virus group because of the properties it shares with SBMV [3]. However, these data indicate that LTSV has a RNA complement consisting of virus- and viroid-like RNA components similar to those of VTMoV, SNMV and SCMoV [4–6]. Hence, it is unlike SBMV which contains no viroid-like RNA. We support the view that a new virus group

should be defined to include viruses with particles like those of SBMV, but which contain viroid-like RNA. Such a group would, at present, include VTMoV, SNMV, SCMoV and LTSV. Of these, VTMoV and SNMV are closely related serologically [4], but should be considered as distinct viruses since their heterologous virus- and viroid-like RNAs are incompatible [8]. SCMoV and LTSV are only distantly related serologically, but neither appears to be related to VTMoV or SNMV [5]. However, their physical properties leave little doubt that they should be included in the same group. To date, all the viruses containing viroid-like RNAs have been found in Australasia.

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