The Middle Component of Strawberry Latent Ringspot Virus

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SUMMARY

A hitherto uncharacterized nucleoprotein sedimenting at 95S to 99S has been observed in preparations of six different isolates of strawberry latent ringspot virus when centrifuged in sucrose-density gradients. This component had the same coat protein composition, serological properties, size and outward aspect of particles as the rest of the virus and encapsidated a molecule of RNA-2, thus exhibiting all major characteristics of the middle (M) component of nepoviruses. One of the virus isolates under study, as well as two functional RNA species, appeared to possess a third satellite-like RNA with mol. wt. $0.45 \times 10^6$.

Strawberry latent ringspot virus (SLRV), a tentative member of the nepovirus group (Harrison & Murant, 1977), differs from other nepoviruses because: (i) the particles contain two polypeptide species (mol. wt. 29000 and 44000) instead of a single polypeptide with a mol. wt. of about 55000; (ii) the middle (M) component, i.e. a nucleoprotein encapsidating a molecule of the smaller RNA species (RNA-2), is not produced (Mayo et al., 1974). However, SLRV strains recently isolated in Italy from olive appeared to possess a nucleoprotein with physicochemical properties typical of those of an M component (Savino et al., 1979). This prompted us to investigate the occurrence of a comparable nucleoprotein in other SLRV isolates of different origin and its biological significance.

A comparative study was carried out using: (i) three isolates from peach (SLRV-Lo, SLRV-P and SLRV-Ax) from northern Italy; (ii) two isolates from olive (SLRV-S and SLRV-11D) from central Italy; (iii) an isolate from strawberry (SLRV-H) (Mayo et al., 1974), kindly supplied by Dr A. F. Murant. All virus isolates were cultured in Chenopodium quinoa Willd. Plant extracts were clarified with magnesium-activated bentonite, virus components fractionated and their RNAs analysed by polyacrylamide gel electrophoresis as described in detail by Savino et al. (1979). RNA preparations were heated at 60 °C in 8 M-urea before polyacrylamide gel electrophoresis. The concentration of each RNA in each inoculum was adjusted to 4 μg/ml in the same final volume. Coat protein was dissociated by heating unfractionated virus or separated M and B components at 100 °C for 3 min in the presence of 0.05 M-tris-HCl buffer pH 7 containing 5% 2-mercaptoethanol (2-ME) and 2% SDS (Laemmli, 1970). Some preparations of SLRV-Lo were heated at 37 °C for 3 h, 60 °C for 30 min or 100 °C for 2 min in 0.125 M-tris-HCl buffer pH 6.8 containing 1% SDS, 2% 2-ME and 6 M-urea (Chu & Francki, 1979). Antisera were prepared to unfractionated SLRV-Lo, the most readily purified strain (up to 4 mg virus per 100 g of tissue, i.e. 0.8 mg for SLRV-H and about 2 mg for other isolates) and to its M and B components separated with three successive cycles of density-gradient centrifugation. The rabbits were given one intramuscular and three intravenous injections (1 ml/injection) at weekly intervals, each containing 0.5 mg nucleoprotein and were bled 15 days after the last injection.

In density-gradient centrifugation, all virus isolates showed a third component (M) sedimenting between the two components (T and B) known to occur in SLRV (Mayo et al., 1974). In peach and olive isolates the intermediate component was apparently homogeneous as indicated by the single sharp peak observed in u.v. absorption traces after sedimentation in sucrose gradients (Fig. 1). Conversely, M component of the strawberry isolate was
heterogeneous showing the tendency to separate into three subcomponents ($M_1$, $M_2$ and $M_3$) sedimenting very close to one another (Fig. 1d). This was a consistent feature of SLRV-H, which did not change with the season of year when the virus was propagated.

The three centrifugal components were variously represented in different isolates (Fig. 1). However, their relative proportions in each strain did not change significantly during the year and were not related to the origin (i.e. natural host) of the virus. As shown in Fig. 1, isolates from the same host plant differed considerably from one another in this respect. Isolates SLRV-Lo, SLRV-S and SLRV-H were examined by analytical centrifugation. As with sucrose density-gradients, SLRV-Lo and SLRV-S sedimented as three components with estimated sedimentation coefficients ($s_{20,w}$) of 62S (T), 99S (M), 135S (B) and 60S (T), 95S (M), 128S (B) respectively. On the other hand, in SLRV-H preparations four components were resolved, sedimenting at 60S (T), 94S (M), 109S ($M_1$) and 135S (B).
Purified unfractionated preparations of all isolates yielded consistently two polypeptides with mol. wt. of 28,500 (±1500) and 44,600 (±1500) (average of 22 determinations) respectively in discontinuous slab gel electrophoresis (Laemmli, 1970) irrespective of the dissociation procedure used. Two polypeptides with a comparable mol. wt. were also obtained when isolated M and B fractions were dissociated and electrophoresed separately. The same two protein species were resolved by co-electrophoresing mixtures of T + M, M + B and T + M + B of SLRV-Lo. Electrophoresis of nucleic acid from unfractionated preparations of all isolates in polyacrylamide tube gels under non-denaturing conditions resolved two RNA species with a mol. wt. of 1.6 (±0.046) × 10^6 (RNA-2) and 2.6 (±0.023 × 10^6 (RNA-1) (average of 15 determinations). Separated B fractions of all isolates contained both RNA species, whereas only RNA-2 was obtained from M fractions. A third RNA species (RNA-3) with mol. wt. of about 0.45 × 10^6 was found in unfractionated preparations of SLRV-H. Substantial amounts of this RNA were present in preparations of M component together with RNA-2 (Fig. 2b). The small amount of RNA-1 found in M component (Fig. 2a) and of RNA-3 in B component (Fig. 2b) of SLRV-H is probably due to contamination of these fractions which, owing to the heterogeneity of M, could not be completely separated.

Infectivity assays done with nucleic acid from SLRV-Lo inoculated on C. quinoa or Cucumis sativus L., indicated that the two RNA species are complementary, neither of them being infective alone. RNA-2 from B or M fractions was equally effective in restoring infectivity when mixed with RNA-1. Infectivity indices (Raymer & Diener, 1969) were 15.5 and 15 (mean of three experiments) for mixtures of M-RNA_2 + RNA_1 and B-RNA_2 + RNA_1 respectively. A virus with three centrifugal components (T, M and B), serologically identical to the original SLRV-Lo, was isolated from plants inoculated with both types of RNA mixtures. The antisera to unfractionated SLRV-Lo, and to M and B components, each had a titre of 1:512. These antisera reacted with M and B antigens of the same isolate and to the same dilution endpoint with precipitin lines that merged at the junction without forming spurs. Moreover, purified M particles were equally well decorated by the antiserum to M component and by antisera to unfractionated virus and to B component in immune electron microscopy tests (Milne & Luisoni, 1975). M particles of SLRV-Lo were morphologically identical to B particles and to M or B particles of other isolates, each having a diameter of about 30 nm, an angular outline and details of the surface structure poorly resolved.

The results of the present study demonstrate that all SLRV isolates under investigation have a class of particles sedimenting between the two reported in the literature of this virus. This intermediate component has the same coat protein composition, serological properties, size and outward aspect as the rest of the virus and comprises coat protein encapsidating a molecule of functional RNA-2. Therefore, it possesses all major characteristics of the M component of nepoviruses with which it can be identified. Hence, in this respect, SLRV does not differ from other members, either tentative or definitive, of the nepovirus group.

In five out of six isolates, M particles sedimented as a single homogeneous class. Heterogeneity was, however, observed in the M component of the strawberry isolate. The reason for this has not been elucidated although it seems likely that the presence of the satellite-like RNA-3 may provide an explanation. The presence of a small RNA species (mol. wt. 0.45 × 10^6) had already been noted in one isolate of SLRV by Mayo et al. (1974), who suggested that it was not essential for virus multiplication. We now confirm the occurrence of a satellite system in SLRV, which resembles that of tomato black ring (Murant et al., 1973) and myrobalan latent ringspot (Delbos et al., 1976; Gallitelli et al., 1981) viruses. The instability of SLRV-H in CsCl prevented a more detailed study of the encapsidation of RNA-3, in relation to the ordinary centrifugal components.

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**REFERENCES**


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