The Protein and Nucleic Acid Components of Elderberry Latent Virus

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SUMMARY

The protein and nucleic acid components of elderberry latent virus (ELV) were of similar size to those of tomato bushy stunt virus (TBSV) when examined by electrophoresis in polyacrylamide gels. Estimates of the mol. wts. of the proteins were 40,000 (ELV) and 42,000 (TBSV), and the estimates of the mol. wts. of the nucleic acids were $1.55 \times 10^6$ (ELV), and $1.8 \times 10^6$ (TBSV). Buoyant densities in caesium chloride were 1.363 g/cm$^3$ (ELV) and 1.348 g/cm$^3$ (TBSV). To account for differences in sedimentation coefficient and in buoyant density between ELV and TBSV, and in comparison with the structure of 180 + 12 protein subunits proposed for TBSV, we suggest that the most likely number of protein subunits in particles of ELV is 120.

INTRODUCTION

Elderberry latent virus (ELV, R/1:*/*:S/S:*S/*) which was found recently in American elder (Sambucus canadensis L.), has an unusual combination of properties which suggests that it is not related to any well-studied group of viruses (Jones, 1972). In a further attempt to determine the affinities of elderberry latent virus to other viruses, we have investigated the mol. wts. of the protein and nucleic acid components of the virus particles. From the results, and comparisons with tomato bushy stunt virus (TBSV) which resembles ELV in some properties (Jones, 1972), we suggest that ELV particles contain 120 protein subunits.

METHODS

Preparation of virus. ELV was extracted from inoculated leaves of Chenopodium quinoa Willd. using 0.06 M-phosphate buffer, pH 7, and purified by chloroform clarification, followed by acid precipitation of the virus and two cycles of differential sedimentation. TBSV (type strain) was extracted from inoculated leaves of C. quinoa or, in some instances, from systemically infected leaves of Nicotiana clevelandii Gray using 0.06 M-phosphate buffer, pH 7, and purified by chloroform clarification and differential sedimentation. Virus preparations were stored in 0.006 M-phosphate buffer, pH 7. In some experiments both ELV and TBSV were further purified by sucrose density gradient sedimentation.

Infectivity assays. Local lesion assays of ELV and TBSV were made in inoculated leaves of Chenopodium quinoa. Because local lesions induced by ELV in C. quinoa leaves are rather indistinct (Jones, 1972), samples were assayed at different dilutions and only results from inocula producing low numbers of lesions were recorded. Inocula were applied on muslin pads to whole leaves (four/plant) previously dusted with carborundum (600 mesh/inch).

Preparation and electrophoresis of RNA. RNA was obtained from purified virus by in-
cubating approximately 0.1% virus in a solution containing pronase (Calbiochem) (1.5 mg/ml), SDS (5 mg/ml), 0.15 M-sodium chloride and 0.015 M-sodium citrate at 37 °C for 16 h. The resulting nucleic acid preparations were either subjected to electrophoresis immediately or recovered by precipitation with 2.5 vol of ethanol. No protein was detected by Coomassie blue staining of acrylamide gels following electrophoresis of these nucleic acid preparations. Electrophoresis was as described by Murant et al. (1972), using 2.5% gels and a current of 6 mA/gel. After electrophoresis for 2 to 3 h, gels were scanned with a u.v. densitometer (UV Scanner, Joyce–Loebi Ltd.). Mol. wts. of RNAs were estimated by comparing their mobilities with those of ribosomal RNAs, of known weight, from pea roots (Loening, 1968).

Preparation and electrophoresis of protein. Polypeptide was prepared for electrophoresis by heating freshly purified virus with 1% SDS + 0.1% dithiothreitol in 0.01 M-sodium phosphate (pH 7) in boiling water for 90 s. Samples were examined directly by electrophoresis in gels of 7.5% acrylamide and 0.2% methylene bisacrylamide in 0.1 M-sodium phosphate (pH 7) + 0.1% SDS, with 0.1% 2-mercaptoethanol in the buffer reservoirs. Current (4 V/cm, 8 mA/gel) was passed for 60 min and samples were then run for 5 h. Gels were stained with Coomassie blue and the mobilities of bands of polypeptide-dodecyl sulphate complexes were measured from microdensitometer (Joyce–Loebi Ltd., Mk III) tracings. Mol. wts. of the virus polypeptides were estimated by comparing their mobilities with those of proteins of known mol. wt. Bovine serum albumin (mol. wt. 66000, Calbiochem Ltd.), ovalbumin (45000), carbonic anhydrase (29000), chymotrypsinogen (25700, Sigma) and tobacco mosaic virus protein (17400) were used as standards. Polypeptide was alkylated by disrupting virus with 8 M-urea + 1% SDS + 0.1% dithiothreitol in 0.15 M-tris-HCl (pH 8.1), making this preparation 0.4 M with iodoacetamide and incubating for 15 min at 37 °C. Polypeptide was recovered by acetone precipitation, dissolved in 0.01 M-sodium phosphate + 1% SDS + 0.1% dithiothreitol and electrophoresed in gels as above.

Preparative sedimentation in caesium chloride. A step-gradient technique for attaining rapid equilibrium was used (Brunk & Leick, 1969). Purified virus was diluted with 0.06 M-phosphate buffer (pH 7.3) to give 1.25 ml with E_{260} of 2.0. Approximately 1.25 ml caesium chloride in 0.05 M-phosphate buffer, density 1.5 g/cm^3, was added and the mixture was layered over 2.25 ml of similar caesium chloride solution. Gradients were centrifuged at 30000 rev/min for 16 h in a Spinco SW 30 rotor at 1 to 5 °C. After centrifuging, 4-drop fractions were collected following upward displacement through an ISCO Model UA-2 ultraviolet analyser (Instrument Specialities Co., Lincoln, Nebraska). The refractive index of alternate fractions was determined at 20 °C in an Abbe refractometer (Bellingham & Stanley Ltd., London) and used to calculate densities. The remaining fractions were diluted and assayed for infectivity.

Analytical sedimentation in caesium chloride. Solid caesium chloride (BDH, for ultracentrifuge work) was added to virus preparations and the resulting density determined with a refractometer. Preparations were centrifuged at 44000 rev/min for 18 h at 5 °C using the AnD rotor of a Beckman Model E analytical centrifuge. Densities were calculated from enlargements of the Schlieren diagrams as described by Szybalski & Szybalski (1971).

Electron microscopy. Purified preparations were examined in a Siemens Elmiskop 1A after negative staining with uranyl formate prepared with sodium hydroxide (I. M. Roberts in Barnett & Murant, 1970).
Protein and RNA components of ELV

RESULTS

Molecular weights of protein

Both ELV protein and TBSV protein migrated as single major components in acrylamide gels (Fig. 1a, b). Although some polypeptide was detected migrating faster than the main bands no clear evidence was obtained for the existence of any minor bands in gels of TBSV protein such as described by Butler (1970), possibly because insufficient material was loaded on the gels. The virus polypeptides were partially resolved in mixtures (Fig. 1c, d) and mol. wt. estimates were ELV, 40000, and TBSV, 42000. These estimates were unaffected when the polypeptides were alkylated before electrophoresis. The estimate for TBSV agrees well with previous estimates, falling into the range of 38000 to 42000 (Butler, 1970; Lesnaw & Reichmann, 1970; Michelin-Laurosot et al. 1970; Weber, Rosenbusch & Harrison, 1970; Carpenter, Cook & Gibbs, 1971; Hull, 1971; Hill & Shepherd, 1972).
M. A. MAYO AND A. T. JONES

(a)

(b)

(c)

Distance →

Distance →

Fig. 2. U.v. densitometer traces of 2-5% acrylamide gels following electrophoresis of nucleic acid samples: (a) elderberry latent virus RNA; (b) tomato bushy stunt virus RNA; (c) a mixture of samples (a) and (b); (d) a separate experiment showing a mixture of elderberry latent virus RNA, tomato bushy stunt virus RNA and nucleic acids from root tissue of pea; peak (1) DNA, (2) tomato bushy stunt virus RNA, (3) elderberry latent virus RNA, (4) large ribosomal RNA, mol. wt. $1.3 \times 10^6$, (5) small ribosomal RNA, mol. wt. $0.7 \times 10^6$. Electrophoresis was in 0-02 M-tris-phosphate buffer (pH 7-8) containing 0-002 M-ethylene diamine tetra-acetic acid and 0-2% SDS. Migration was from left to right.

Molecular weights of nucleic acid

Nucleic acids of ELV and TBSV migrated as single electrophoretic components (Fig. 2a, b). Mixtures of the virus nucleic acids were resolved (Fig. 2c), and both virus nucleic acids were resolved from nucleic acids of pea root (Fig. 2d). Mol. wt. estimates were $1.55$ and $1.8 \times 10^6$ for ELV and TBSV respectively. This value for the mol. wt. of TBSV RNA compares with other estimates of $1.55 \times 10^6$ (De Fremery & Knight, 1955) and $1.6$ to $1.7 \times 10^6$ (Tremaine, 1970) calculated from the percentage RNA and the particle weight, and a recent estimate of $1.65 \times 10^6$ based on sedimentation and electrophoresis in polyacrylamide gels (Dorne & Pinck, 1971).

Buoyant density in caesium chloride

When preparations of ELV were centrifuged in caesium chloride in the preparative ultracentrifuge a substantial proportion of the virus was degraded to form a band of highly aggregated material of density 1.30 to 1.32 g/cm$^3$. No virus particles were detected in this band. When ELV was purified by sucrose density-gradient sedimentation the proportion of virus degraded by caesium chloride was increased; thus for equilibrium sedimentation experiments only differential sedimentation was used to purify ELV. Undisrupted virus particles formed a band of density 1.37 to 1.38 g/cm$^3$ (two determinations), which corresponded with fractions of greatest infectivity (Fig. 3a).

TBSV preparations also formed a band of aggregated material of density 1.29 to 1.30 g/
Protein and RNA components of ELV

Fig. 3. Preparative sedimentation in caesium chloride. Preparations were centrifuged in step gradients for 16 h at 30000 rev/min in a Spinco SW 39 rotor. The absorptiometer traces (solid line) are shown together with the densities (●—●—●) and infectivities (solid bars) of alternate 4-drop fractions. (a) Elderberry latent virus preparation, 1 ml of E₂₆₀ = 5; infectivity is the number of lesions formed per Chenopodium quinoa leaf. (b) Tomato bushy stunt virus preparation, 1 ml of E₂₆₀ = 4; infectivity is total lesions formed in four leaves of C. quinoa.

cm³ when centrifuged in caesium chloride. Virus particles formed a band of density 1.34 to 1.36 g/cm³ (two determinations) which corresponded with the fraction of maximum infectivity (Fig. 3b).

In these experiments infectivity was not symmetrically distributed on either side of the peak fractions (Fig. 3) and this was attributed to the infective material trailing when the gradients were fractionated by upward displacement.

Three density components were resolved in ELV preparations by analytical sedimentation in CsCl (Fig. 4a). The least dense was highly aggregated, and corresponded to the upper zone found in gradients from the preparative centrifuge; the other two corresponded to the zone of maximum infectivity. The densities of the two dense bands were estimated to be 1.363 and 1.372 g/cm³. The less dense corresponds to the absorbance peak containing virus in Fig. 3(a), although either density component could correspond to the peak of infectivity. Minor density components were recently reported in isolates of tobacco rattle virus and these were lost when the isolates were cloned by single lesion culture (Cooper & Mayo, 1972). It is possible that the minor component of ELV of density 1.372 g/cm³ is a similar variant, and the density of the predominant strain of ELV was therefore 1.363 g/cm³. This value is used in subsequent calculations.

TBSV which had been purified by sucrose density-gradient sedimentation comprised a single component of density 1.348 g/cm³ in CsCl density gradients (Fig. 4b). This component corresponds to the absorbance peak containing virus and infectivity in Fig. 3(b).
Fig. 4. Analytical sedimentation at 44,000 rev/min; Photographs taken after centrifuging for approximately 18 h at 5 °C, (a) elderberry latent virus in caesium chloride in 0.035 M-phosphate buffer (pH 7.3) initial density 1.366 g/cm³, \( E_{260} = 7 \), Schlieren angle 65°. (b) tomato bushy stunt virus in caesium chloride in 0.017 M-phosphate buffer (pH 7.3) initial density 1.329 g/cm³, \( E_{260} = 1 \), Schlieren angle 55°.

**DISCUSSION**

ELV resembles TBSV in that we were unable to resolve mixtures of the viruses into different diameter classes by electron microscopy and the sizes of the protein and nucleic acid components of the two viruses are similar. However, the viruses have widely different sedimentation coefficients (\( s_{20, w} \)), 112 S for ELV (Jones, 1972) and 133 S for TBSV (Dorne & Pinck, 1971), and have different buoyant densities in caesium chloride. We suggest that these differences are due to the different weights of protein and nucleic acid in the virus particles.

Sehgal et al. (1970) derived an empirical relationship between the percentage nucleic acid in a virus particle and its buoyant density in caesium chloride. Estimates of the percentage RNA obtained by substituting our values for buoyant densities into this formula are ELV, 23.0 % and TBSV, 19.7 %. From these data and the estimates of nucleic acid mol. wts. the estimated particle mol. wts. are ELV, \( 6.7 \times 10^6 \) and TBSV, \( 9.1 \times 10^6 \). If a probable error of \( \pm 0.1 \times 10^6 \) is assumed for the estimate of nucleic acid mol. wt. these estimates of particle mol. wt. are \( \pm 0.4 \times 10^6 \). Previous estimates of the percentage RNA composition of TBSV were 17 to 20 % (De Fremery & Knight, 1955; Ambrosino et al. 1967; Tremaine, 1970). If, therefore, our figures are overestimates by about 2 % RNA the values become 18 % RNA (TBSV) and 21 % (ELV), and using these figures, particle mol. wt. estimates would be increased to 7.4 and \( 10 \pm 0.4 \times 10^6 \) for ELV and TBSV, respectively.
An alternative estimate of particle mol. wt. can be obtained by substituting the sedimentation coefficient into the Svedberg equation (Markham, 1967). Using the value for the diffusion coefficient determined for TBSV, $1.26 \times 10^{-7} \text{ cm}^2/\text{s}$ (Schachman & Williams, 1959; Weber et al. 1970), assuming partial specific vol. of 0.55 and 0.74 for RNA and protein, respectively (Markham, 1967) and 19.7% RNA, the estimated particle mol. wt. for TBSV is $8.6 \times 10^6$. Estimates of the dry diameter of the viruses were similar, therefore if ELV particles are hydrated to the same extent as those of TBSV the diffusion coefficients will be the same. Using this assumption and a value of 23% RNA, the estimated particle mol. wt. of ELV, using the Svedberg equation, is $7.1 \times 10^6$.

Irrespective of the absolute values, there is a difference between the estimates of particle mol. wts. for the two viruses of 1.5 to $3.0 \times 10^6$. Estimates of the mol. wts. of nucleic acids indicate that TBSV RNA is $0.25 \times 10^6$ larger than that of ELV RNA, and thus the difference between the mol. wt. of protein in TBSV particles and in ELV particles is 1.25 to $2.75 \times 10^6$.

TBSV particles have been found to comprise 180 protein subunits of approximately 40000 mol. wt. and 12 protein subunits of 28000 mol. wt. (Butler, 1970; Hill & Shepherd, 1972). The difference in particle mol. wt. between the viruses represents the 12 minor protein subunits plus between 23 and 60 ELV protein subunit molecules of 40000 mol. wt. The estimated number of protein subunits in ELV particles is therefore 157 to 120. If the virus structure conforms to icosahedral symmetry the virus particle should comprise an integral multiple of 60 asymmetric units (Klug & Caspar, 1960), and we therefore suggest that the most likely structure of ELV is 120 protein molecules per particle.

A structure based on 60 asymmetric units each consisting of two unequal sized polypeptides has been proposed for particles of cowpea mosaic virus (Wu & Bruening, 1971). We suggest that the structure of ELV is similar but with the polypeptides being about the same size or possibly identical. No evidence of morphological sub-units was found in electron micrographs, and it is not possible to predict their arrangement because the appearance of the structure we suggest can vary widely depending on the relative separation and the shape of the two parts of the asymmetric unit (Klug & Caspar, 1960).

In the absence of an experimentally determined particle mol. wt. for ELV it is not possible to confirm the particle structure we suggest. However, our evidence tends to support the suggestion by Jones (1972), based on serology and biological properties, that ELV is not closely allied to any well characterized virus or group of viruses. It may therefore be best classified as a member of a monotypic virus group (Harrison et al. 1971).

The revised cryptogram for ELV is $R/1:1.55/(23):S/S:/S/*$.

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REFERENCES


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