

PURIFICATION AND SOME PROPERTIES OF *CENTROSEMA* MOSAIC VIRUS

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Summary

A method is described for the purification of *Centrosema* mosaic virus from leaves of *Crotalaria anagyroides* H.B.K. Partially purified preparations contained elongated flexuous rods, measuring approximately 580 by 12 m μ , which were regularly associated with infectivity. These preparations had an ultraviolet absorption spectrum characteristic of a nucleoprotein and showed only a single homogeneous peak when examined in the analytical ultracentrifuge. The identity of the virus is discussed.

I. INTRODUCTION

Centrosema mosaic was recently described (Van Velsen and Crowley 1962) as a virus disease of *Centrosema pubescens* Benth. and *Crotalaria* spp. in Papua and New Guinea. The host range and vector studies already reported indicated that *Centrosema* mosaic was caused by a previously undescribed virus which produced similar symptoms to those of *Crotalaria* mosaic virus (Das Gupta, De, and Raychaudhuri 1951), to a strain of tobacco mosaic virus (Bawden 1956), and to a strain of cowpea mosaic virus (Anderson 1955).

In this paper it is shown that *Centrosema* mosaic is regularly associated with the presence of elongated, flexuous, rod-shaped particles. A method for the partial purification of *Centrosema* mosaic virus (CaMV) is described.

This finding supports the data of Van Velsen and Crowley (1962) which indicated that this virus had not previously been described.

II. MATERIALS AND METHODS

The CaMV isolate used was identical with that studied by Van Velsen and Crowley (1962). Plants of *Crotalaria anagyroides* H.B.K. were inoculated when at the cotyledonary stage (7-10 days after sowing) with infectious sap mixed with 0.1M phosphate buffer, pH 7. Symptoms developed on the trifoliate leaves about 2 weeks after inoculation and leaves were harvested within 2 weeks after the symptoms appeared. As no local lesion host is available, all infectivity assays were carried out by inoculating at least 20 young (7-10-day-old) seedlings of *C. anagyroides*.

Optical densities of virus preparations were measured in a Shimadzu spectrophotometer. A cell of 1 cm path length was used in these measurements.

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III. EXPERIMENTAL AND RESULTS

(a) *Partial Purification of CaMV*

The method finally adopted was essentially similar to that used by Steere (1956) for the purification of tobacco ringspot virus. Each 100 g of leaf material from plants showing disease symptoms was homogenized in a high-speed "Atomix" Blender together with 50 ml 0.5M phosphate buffer, pH 7, 100 ml chloroform-n-butanol mixture (1 : 1 v/v), and 10 ml 0.5M ascorbic acid. The macerate was strained through cheese

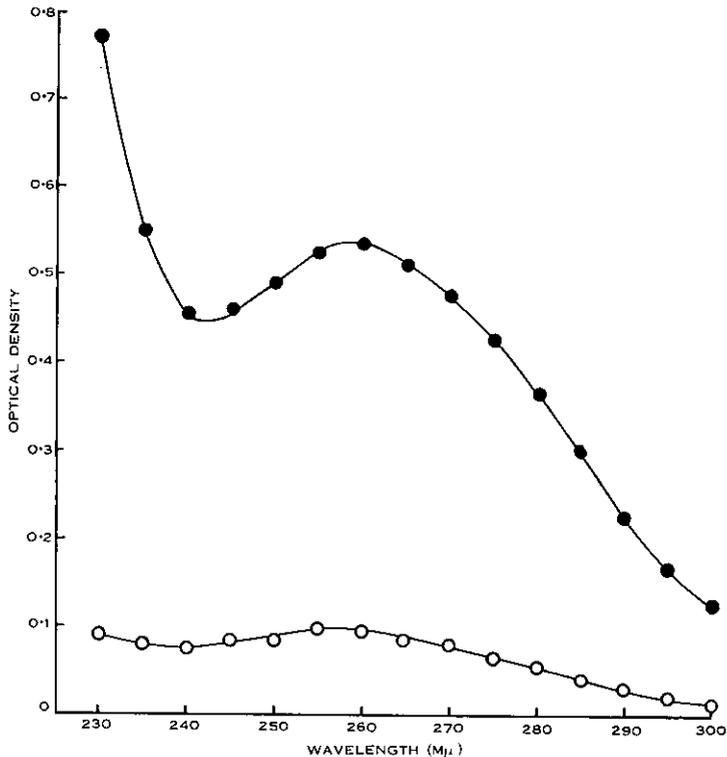


Fig. 1.—Ultraviolet absorption spectrum of a partially purified preparation of *Centrosema mosaic virus*. ● Preparation from infected leaves. ○ Preparation from an equivalent amount of healthy material.

cloth and centrifuged at 3000 r.p.m. for 10 min. The buffer layer was decanted, kept for 30 min, and centrifuged at 10,000 r.p.m. in a No. 30 rotor of a Spineo model L ultracentrifuge. Virus was then sedimented from the supernatant by centrifugation for 1 hr, in either a No. 30 or No. 40 rotor. The pellet was resuspended in 0.1M phosphate buffer at pH 7 and the process of alternate low- and high-speed centrifugation was repeated twice more. All the above operations were carried out at 2–4°C.

The material of the final pellet when suspended in 0.1M phosphate buffer, pH 7, exhibited the following properties:

- (1) It was infectious to *C. anagyroides* for at least 12 days when stored at 2–4°C.
- (2) It possessed an ultraviolet absorption spectrum characteristic of a nucleoprotein, having a maximum at about 259 $m\mu$ and a minimum at about 242 $m\mu$. A similar preparation from an equivalent amount of leaf material from healthy plants showed relatively little absorption in ultraviolet light (Fig. 1).
- (3) When centrifuged at 9945 r.p.m. in the Spinco model E analytical ultracentrifuge (An D rotor), a single homogeneous peak was observed. The virus had an uncorrected sedimentation coefficient of approximately 150 S. No peak could be detected in a similar preparation made from healthy leaf material.
- (4) On examination in the electron microscope the preparation from virus-infected leaves was observed to contain elongated flexuous rods (Plate 1, Figs. 1 and 2) which were absent in the preparation made from uninfected leaves.

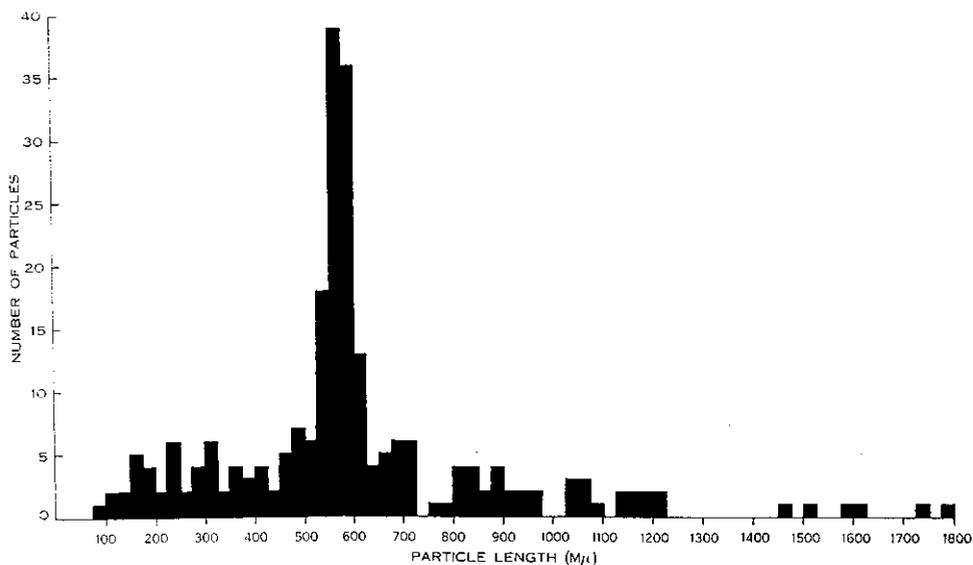


Fig. 2.—Distribution of particle lengths of elongated flexuous rods of *Centrosema* mosaic virus which has been partially purified by two cycles of ultracentrifugation.

(b) Particle Length of CaMV

Partially purified virus preparations were sprayed onto electron-microscope grids coated with a film of collodion and backed with a film of carbon. The mounts were shadowed with palladium and examined with a Philips "Metalox 100" electron microscope. Lengths of the elongated particles were measured by projecting electron-micrograph negatives onto a screen, tracing the particles on drawing paper, and then fitting pieces of 1.5 mm diameter plastic tubing along their length. Widths of the particles were measured from grids onto which preparations of both CaMV and tobacco mosaic virus were sprayed, the width of the latter being taken as 15 $m\mu$.

The flexuous, rod-shaped particles were $12 \pm 2 \text{ m}\mu$ in diameter and in our preparations were found in various lengths (Fig. 2). Out of 237 particles measured 18% were shorter than $400 \text{ m}\mu$ and 17% were longer than $800 \text{ m}\mu$. The average length of the particles was $600 \text{ m}\mu$ when all particles were taken into account, and was $580 \text{ m}\mu$ when rods under $400 \text{ m}\mu$ (probably the result of fragmentation) and over $800 \text{ m}\mu$ (probably the result of aggregation) were excluded. From these data it is concluded that the elementary particle length of CaMV is $580 \pm 20 \text{ m}\mu$.

(c) Concentration of CaMV in Infected Leaves

It appears that the concentration of CaMV is very low in infected leaves which showed pronounced mosaic symptoms. Our evidence for this statement is as follows:

- (1) After partial purification of the virus by the method described above, very low yields of nucleoprotein were obtained. For example when 700 g of leaf tissue showing pronounced disease symptoms were used for making 2.5 ml of a partially purified preparation the optical density of this suspension at $260 \text{ m}\mu$ was only 6.4. Assuming that the virus has a similar nucleic acid content to that of tobacco mosaic virus the yield of CaMV would have been less than 5 mg.
- (2) No peak with a sedimentation coefficient similar to that of CaMV could be detected when freshly extracted infectious sap was examined in the analytical ultracentrifuge.

IV. CONCLUSION

Van Velsen and Crowley (1962) suggested, on the basis of host range, physical properties, and vector transmission that CaMV had not been previously described. The investigations reported here support this conclusion. CaMV is a long flexuous rod whereas previously reported mosaic diseases of *Crotalaria* are caused by the following viruses:

- (1) *Crotalaria* mosaic virus, which has a spherical particle 26–40 $\text{m}\mu$ in diameter (Das Gupta, De, and Raychaudhuri 1951);
- (2) a strain of tobacco mosaic virus (Bawden 1956);
- (3) cowpea mosaic virus (Anderson 1955), which has a particle length about half the length of the virus reported in this paper (Brandes 1959) and differs markedly in host range and physical properties (Van Velsen and Crowley 1962).

It appears that CaMV occurs in very low concentrations in infected leaves of *Crotalaria* plants even though pronounced mosaic symptoms are readily produced. Any future studies of the properties of this virus will, therefore, be limited by the difficulty of obtaining sufficient partially purified material.

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Figs. 1 and 2.—Electron micrographs at different magnifications of *Centrosema* mosaic virus which has been partially purified by two cycles of ultracentrifugation. The polystyrene latex particles are $110\text{ m}\mu$ in diameter.

