**In vitro Degradation Products of Tobacco Rattle Virus**

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**SUMMARY**

When particles of tobacco rattle virus (TRV) were progressively degraded by alkali, detergent or urea a number of particles of specific lengths were produced. Some of the breakdown products had lengths similar to those of the naturally occurring short rods characteristic of various isolates of TRV. Loss of infectivity was associated with increasing heterogeneity of sedimentation of the long TRV particles during density gradient centrifugation. Cleavage of different strains of TRV produced relatively few fragments from 1800 to 1900 Å resembling the naturally occurring shorter rods.

**INTRODUCTION**

Tobacco rattle virus (TRV) has been shown to exist as a number of variant forms possessing characteristic particle length distributions (Harrison & Woods, 1966). Speculation on the role of the noninfectious shorter rods (Harrison & Nixon, 1959) has centred on their being either a precursor or degradation product of the infectious long rods or a discrete product with distinct functions (Lister, 1966). The present understanding of protein and nucleic acid synthesis and virus fabrication does not support the precursor hypothesis. Also, because the short rods produced by any one strain do not have lengths, some multiple of which would equal a complete infectious rod length, the degradation product hypothesis has been considered implausible. There is, however, preliminary evidence to suggest that the short particles have special functions (Lister, 1966).

More recently, two closely related isolates of TRV, producing particles of three mean lengths and differing only in the length of the intermediate size particle have been purified and characterized (Semancik, 1966). In studying the effect of age of infection on particle length distribution of a strain (c) of tobacco rattle virus from California which produces particles mostly 450 to 550, 800 to 900 or 1800 to 1900 Å long (Semancik, 1966), Semancik & Kajiymama (1967) isolated another fraction (B') 1600 to 1700 Å long from samples taken during the period of most active virus synthesis. Particles of this length were readily broken in half.

This observation prompted a more thorough investigation of the possibility that 1900 Å rods can be broken into specific shorter lengths. *In vitro* degradation of complete rods of tobacco mosaic virus with the accompanying production of stable fragments has been accomplished by treatment with alkali (Harrington & Schachman, 1956), detergent (Hart, 1955), and urea (Buzzell, 1960, 1962). In this paper we describe some effects of these agents on the lengths and infectivity of TRV.
METHODS

Virus preparation and assay. Strains B and C of tobacco rattle virus characterized by particle lengths of 450 to 550, 1050 to 1150 and 1750 to 1900 Å for B and 450 to 550, 800 to 900 and 1750 to 1900 Å for C were propagated in Nicotiana clevelandii (Gray) and purified by the method of Semancik (1966) excluding the precipitation at pH 5.0. Local lesion assays of infectivity were made on bean (Phaseolus vulgaris L. var. The Prince) in an incomplete block design with at least 8 half-leaves per treatment. Because strain C produced particles 1600 to 1700 Å long early in infection this isolate was used in most of the experiments.

Before and after treatment under various conditions, preparations were centrifuged in a Spinco SW25.1 rotor for 1½ to 2 hr at 24,000 rev./min. in a preformed density gradient column of 100 to 400 mg./ml. sucrose in 0.1 M-potassium phosphate buffer, pH 7.0. After centrifugation the gradients were examined using an ISCO (Instrumentation Specialites Co., Lincoln, Nebraska, U.S.A.) density gradient fractionator and ultraviolet (254 m/~) analyser and samples were collected.

Reaction conditions. The amount of virus used for each treatment (0.5 to 1.5 mg.) was adjusted on the basis of readings of optical density at 280 mµ. Virus was degraded by alkali by dialysing preparations for 18 hr at 4° against 0·1 ionic strength buffers from pH 10·0 to 11.0 (Miller & Golder, 1950). Degradation products were separated in pH 10·0 density gradient columns.

Virus was degraded using 0·05 to 0·3 % (w/v) sodium lauryl sulphate by treating preparations for 2 to 15 min. at 37°. The reaction was stopped by adding 0·1 ml. of 1 M-KCl to the 1.5 ml. reaction mixture and immersing in an ice bath for 5 min. The precipitated detergent was removed by centrifuging at 3400 rev./min. (Adams safeguard centrifuge) for 5 min.

Treatment with urea (6M) was for 5 to 40 min. at 0 to 10°. The reaction was stopped by adding 2 vol. of 0·02 M-phosphate buffer, pH 7·5, and centrifuging immediately in sucrose density gradients.

Electron microscopy. Droplet samples from the density gradient fractionator were placed on Formvar + carbon coated grids for about 2 min., then repeatedly rinsed with droplets of deionized water. Excess liquid was removed after each application by touching the grid with filter paper. The grids were then shadowed at an angle of 4:1 with palladium and viewed in a modified RCA Model EMU-3B electron microscope. Particle lengths were measured from electron micrographs made at a magnification of 20,000 and enlarged 10 times.

RESULTS

Degradation by alkali

No effects of buffers of pH 7·0 to 10·0 on isolate C were detected by density gradient analysis, but changes in pH of 0·1 to 0·2 units between pH 10·0 and 11·0 induced large changes (Fig. 1). Treatment at pH 11·0 resulted in the loss of virus-like material sedimenting at normal rates and the appearance of much ultraviolet absorbing material at the meniscus of the gradients, indicating that all rod-like structures had disappeared. Infectivity, likewise, decreased greatly with increase of pH value between 10·0 and 10·9 (Fig. 2).

The changes of scanning patterns presented in Fig. 1 can arbitrarily be divided into
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discrete stages to aid interpretation. From pH 10.0 to 10.5 the most obvious effect was of the apparent conversion of the native middle component (800 to 900 Å) to a shorter 600 to 700 Å (M') particle. The bottom component (1800 to 1900 Å) appeared less affected by these conditions because it gave only a slightly broader band in density gradients. At pH 10.7, the 600 to 700 Å particle underwent further degradation and

![Diagram](https://via.placeholder.com/150)

Fig. 1. Scanning patterns of alkali-treated TRV-c after rate zonal sedimentation in sucrose density gradients. Exposure to pH 10.0, 10.3, 10.5, 10.7 and 10.9 indicated (top to bottom). Direction of sedimentation is indicated by arrow and relative positions of normal components are indicated on the baseline.

particles resembling native top component (450 to 550 Å) and others of 300 to 400 Å, designated T', appeared. Particles 1600 to 1700 Å long, similar to the B' fraction found in recently infected leaves (Semancik & Kajiyama, 1967) also appeared. At pH 11.0 a few particles of 800 to 900 Å appeared, presumably derived from B by way of B'.

Because the conversion at pH 10.5 of particles 800 to 900 Å long to ones 600 to 700 Å long is a subtle change, the possibility that it might be a reversible configurational change was investigated. A portion of a stock preparation kept at pH 7.0 was dialysed for 18 hr at pH 10.5, and another fraction was similarly treated and then dialysed for 24 hr at pH 7.0. Timing was arranged so that the treatments were completed simultaneously and the samples centrifuged in the same rotor in density gradients at pH 7.0. Figure 3 presents scanning patterns of the three samples. The
Fig. 2. Inactivity of TRV-c after exposures to alkali for 19 hr at 4° or after reaction with sodium lauryl sulphate for 5 min. at 37°. Samples were adjusted to an $E_{260} = 0.1$ for pH studies and $E_{260} = 0.08$ for sodium lauryl sulphate treatments.

Fig. 3. Scanning patterns of untreated TRV-c at pH 7.0 (upper), of virus exposed to pH 10.5 (middle), and of virus exposed to pH 10.5 and then dialysed to pH 7.0 (lower) after rate zonal sedimentation in pH 7.0 sucrose density gradients. Direction of sedimentation is indicated by arrow and relative positions of normal components are indicated on the baseline.
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pH 10.5 pattern is converted to a near normal pattern by redialysis at pH 7.0. Infectivity was similarly enhanced by redialysis to pH 7.0. Counts of 197, 36, and 103 lesions/half leaf were obtained with the inocula at pH 7.0, 10.5, and 10.5 redialysed to pH 7.0, respectively. All were used at an $E_{260} = 0.1$.

Fig. 4. Scanning patterns of TRV-c treated with sodium lauryl sulphate after rate zonal sedimentation in sucrose density gradients. Progressive degradation is indicated by the change from the normal pattern after exposure to various combinations of sodium lauryl sulphate concentration (0.05 to 0.3%) and reaction times (2 to 15 min.); from top to bottom: untreated, 0.06% 5 min., 0.13% 15 min., 0.3% 2 min., 0.15% 5 min., 0.3% 4 min., 0.2% 5 min., 0.3% 8 min. Direction of sedimentation is indicated by arrow and relative positions of normal components are indicated on the baseline.

Fig. 5. Scanning patterns of TRV-b treated with sodium lauryl sulphate after rate zonal sedimentation in sucrose density gradients. Progressive degradation is indicated by the change from the normal pattern after exposure to various combinations of sodium lauryl sulphate concentrations (0.15 and 0.3%) and reaction times (2 to 8 min.); from top to bottom: untreated, 0.15% 2 min., 0.15% 4 min., 0.3% 3 min., 0.3% 5 min., 0.3% 8 min. Direction of sedimentation is indicated by arrow and relative position of normal components is indicated on the baseline.

Upon closer inspection of the pattern given by the stock preparation at pH 7.0, a small amount of u.v. absorbing material can be seen that sediments like particles 600 to 700 Å long. The virus treated first at pH 10.5 and then dialysed to pH 7.0 does not give a pattern identical to that given by untreated virus, especially in the M' region, suggesting that extra particles of 600 to 700 Å are present. Hence, the M' form could appear to comprise either a native length which is made more apparent at pH 10.5 or a
configurational variant of the 800 to 900 Å particles that is favoured at pH 10.5 perhaps an effect similar to the pH-induced radial expansion observed with bromegrass mosaic virus (Incardona & Kaesberg, 1964).

Fig. 6. Length distributions of degradation intermediates of TRV-c isolated after rate zonal sedimentation in sucrose density gradients. All fractions were obtained by degradation with sodium lauryl sulphate with the exception of M' from M, obtained by degradation at pH 10.5. From top to bottom: (A) Normal TRV-c with addition of TRV-a middle component (diagonal line), (B) B' from B, (C) TRV-c and TRV-a middle from B', (D) M' from M, (E) M' and T from B' and (F) T' from M.
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Degradation by detergent

Early stages of the breakdown of particles of isolate c corresponded to those of alkali degradation, with the appearance of M' and disappearance of M (Fig. 4). The disappearance of B (1800 to 1900 Å) and appearance of B' (1600 to 1700 Å) and T' (300 to 400 Å) was more clearly indicated after treatment for 4 min. with 0.3% sodium lauryl sulphate. After treatment for 8 min. with 0.3% sodium lauryl sulphate, particles resembling native M particles (800 to 900 Å) and a mixture of T (450 to 550 Å) and M' (600 to 700 Å) were found. Exposure to 0.1 to 0.25% detergent for 5 min., the most effective conditions for particle degradation, also decreased infectivity most rapidly (Fig. 2).

Degradation of particles of isolate b, which produced longer intermediate particles (1050 to 1150 Å) than isolate c, gave particularly characteristic nucleoprotein fragments (Fig. 5). Particles of 1050 to 1150 Å disappeared and those of 300 to 400 Å shorter length appeared, as with the isolate c. More notable, however, was the appearance after exposure to 0.3% sodium lauryl sulphate for 8 min. of particles sedimenting in the region of native middle particles. These particles of 1050 to 1150 Å seemed to have been derived from native particles of 1800 to 1900 Å.

Degradation by urea

Urea (6m) degraded shorter particles of isolate c more drastically than infectious ones. After complete breakdown of the middle moiety, the bottom particles were degraded to particles sedimenting similarly, although more heterogeneously than native middle length particles.

Relationship of degradation products to native particle population

Exposing TRV to degrading agents produced a number of specific nucleoprotein fragments. The distributions of particle lengths presented in Fig. 6 represent a summary statement of the particle lengths that appeared representative portions of degraded preparations. The distributions of normal particle lengths of both the b and c isolates are included for comparison. Except for lengths of particles in the M' fraction obtained by treatment with alkali, all other histograms were obtained from samples treated with sodium lauryl sulphate.

Discussion

The methods used for fragmentation of purified rods of TRV were chosen because of their different modes of primary action. These probably involve either an increase in electrostatic repulsion between protein subunits or a weakening of hydrophobic and hydrogen bonds. Studies with tobacco mosaic virus have shown that alkaline degradation at pH 9.8 can give nucleoprotein rods of about one third of the usual length (Harrington & Schachman, 1956). Stable particles of two thirds to one half the usual length of tobacco mosaic virus were obtained by action of 6 M-urea (Buzzell, 1960). Evidently partial degradation produces particles of specific lengths, and these lengths are not found as representative portions of undegraded preparations.

Tobacco rattle virus strains produce relatively large numbers of noninfectious short rods. We have attempted to see whether such rods could be produced from longer ones by in vitro degradation. We found, first, that short rods were more susceptible than the complete rods to degradation by all agents used, and secondly, that the particles...
formed by fragmentation were of specific lengths, some of which corresponded to lengths reported for one or more strains of TRV (Harrison & Woods, 1966). With the isolate c, stable degradation intermediates of 300 to 400, 600 to 700 and 1600 to 1700 Å designated T', M' and B' were produced, apparently by removal of a 200 to 300 Å portion from the naturally occurring T, M and B lengths. However, the complete infectious length particle was not efficiently converted to the shorter length characteristic of the particular strain. This evidence does not exclude the possibility that naturally occurring short rods are derived from longer ones because the mechanisms of degradation operating in our tests may differ from those occurring in vivo.

Different strains of TRV produce different characteristic lengths of short particles. Assuming that the protein subunits distributed throughout a particle are identical, the different lengths of recoverable short rods are probably not due to the protein-protein bonding but to the firmness of binding of the protein to different parts of the polynucleotide chain (Caspar, 1963). Since strains can display differences in their nucleotide sequence (Symons et al. 1963), the parts that bind most strongly may be distributed differently along the polynucleotide chains of different isolates. This would perhaps produce distinct susceptible sites in the RNA of TRV strains which would be reflected in production of characteristic lengths of short rods. Another, not mutually exclusive, possibility is that some parts of the polynucleotide chain are more stable than others and that some of the completed chains are degraded to relatively stable fragments before they become coated with virus protein.

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REFERENCES


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