Evidence for the Release of 28 S RNA from Turnip Yellow Mosaic Virus Heated in vitro

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The RNA obtained by heating turnip yellow mosaic virus at about 40° was found by Lyttleton & Matthews (1958) to sediment at 2 to 5 S. Such RNA would almost certainly be extensively broken down and non-infectious, for there is much evidence that whole, infectious RNA from turnip yellow mosaic virus sediments at 28 S (Haselkorn, 1962; Dunn & Hitchborn, 1966). However, Kaper & Steere (1959) found that the RNA obtained from turnip yellow mosaic virus by heating was, in fact, infectious, which suggests that degradation of the RNA is not intrinsic to the process of disruption of the virus by heat. The experiments we describe were made in an effort to obtain further information on this point. In particular, the effect of taking precautions to reduce ribonuclease action on the released RNA was examined.

All the work was done using the necrotic RADEMACHER strain (Dunn & Hitchborn, 1966) of the virus grown in Chinese cabbage (Brassica chinensis L). As there is evidence that degradation of RNA may take place within stored turnip yellow mosaic virus particles (Haselkorn, 1962) all experiments were made on freshly purified virus. Purification was effected by two methods, both of which were based on the differential centrifugation of sap expressed from ground leaves. The essential difference between the methods was that in one EDTA, and in the other bentonite, was used to facilitate purification. Both methods have been described in detail elsewhere together with evidence that, whereas virus prepared with EDTA has associated ribonuclease activity, virus prepared with bentonite has not (Dunn & Hitchborn, 1965). Virus suspensions, at a concentration of 1 mg./ml, were heated in glass tubes in a water bath, bentonite (1 to 2 mg./ml.), which is known to adsorb and inhibit ribonuclease (Brownhill, Jones & Stacey, 1959; Singer & Fraenkel-Conrat, 1961; Huppert & Pelmont, 1962) being added, suspended in 0.1 M-NaCl, to some samples of virus before heating. The effect of heat on the virus was observed by centrifuging in a Spinco Model E analytical ultracentrifuge. All sedimentation coefficients are given as the values at 20°. The measures taken to minimize contamination of apparatus and containers with ribonuclease were the same as those adopted during other work (Dunn & Hitchborn, 1966).

Virus prepared by the two methods was held at 45° for 30 min. in 0.05 M-NaCl + 0.05 M-phosphate buffer, pH 7.4, and their schlieren patterns were compared (Pl. 1, fig. 1). Untreated samples of the two preparations were closely similar (Pl. 1, fig. 1 A) and contained a major fraction sedimenting at 110 S (‘bottom’ component of the virus) and a minor 50 S fraction (‘top’ component) (Markham, 1951). Heating virus prepared with EDTA removed almost all the ‘bottom’ component, slightly increased the size of the ‘top’ component peak and produced some polydisperse material which sedimented at about 35 S (Pl. 1, fig. 1 B). This result resembled that obtained by Lyttleton & Matthews (1958) except that the slow-moving peak observed by them and attributed to turnip yellow mosaic virus RNA was very indistinct in the present experiment. In contrast, virus prepared with bentonite yielded a component sedimenting at
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28 S which was also produced on heating virus prepared with EDTA in the presence of added bentonite (Pl. 1, fig. 1C, D). This component was completely lost when heated ‘bentonite’ virus was incubated with pancreatic ribonuclease (10 μg./ml.) for 2 hr at room temperature, the schlieren pattern produced by the suspension then resembling that shown in Pl. 1, fig. 1B. Further observations, using the ultraviolet optics of the ultracentrifuge, were made on suspensions diluted with 0.05 M-NaCl + 0.05 M-phosphate buffer to give $E_{260} = 1$. Untreated ‘bentonite’ virus gave a single sharp boundary representing ‘bottom’ component (Pl. 1, fig. 2A) which microdensitometer traces showed contributed more than 90% of the optical density of the suspension. After heating, this boundary was no longer detectable, instead, some 70% of the total ultraviolet absorbing material sedimented at 28 S and a further 20% at 13 to 28 S (Pl. 1, fig. 2B). If, instead of diluting with 0.1 M salt, heated suspensions were diluted with water so that $E_{260} = 1$ but the salt concentration was 0.01 M, the major component sedimented at 22 S instead of 28 S. When such suspensions were reheated at 65° for 3 min. 50% of the 22 S material was unaffected, the rest appearing to be degraded.

There is considerable circumstantial evidence to support the view that the 22/28 S component produced in the foregoing experiments represents viral RNA. First, there are quantitative considerations. From measurements of the areas under the relevant schlieren peaks it can be calculated that the amount of this component in suspensions of heated ‘bentonite’ virus was equivalent to about three-quarters of the RNA originally present in the virus. From ultraviolet photographs it was estimated that about the same fraction of the optical density at 260 mμ associated with the unheated ‘bottom’ component, and which would be due largely to its RNA (Markham, 1959), was associated with the 22/28 S component after heating. Secondly, the change in the sedimentation coefficient of the material from 28 S to 22 S as the ionic strength of the medium was decreased, parallels the behaviour of RNA from tobacco mosaic virus and turnip yellow mosaic virus under similar circumstances (Haselkorn, 1962; Dunn & Hitchborn, 1966; Boedtker, 1960). Finally, the component appeared to be ribonuclease-sensitive, for it was produced only if virus was heated in an environment presumably free of the enzyme in an active state, and it was eliminated from suspensions on incubation with added enzyme. As a substantial fraction of the RNA was stable at 65°, previous studies (Haselkorn, 1962; Dunn & Hitchborn, 1966) indicate that it may have been infectious, although this was not tested.

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REFERENCES


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EXPLANATION OF PLATE

The effect of heating turnip yellow mosaic virus at 45°C for 30 min. in 0.05 M-NaCl + 0.05 M phosphate buffer, pH 7.4.

Fig. 1. Schlieren patterns produced on analytical centrifugation. (A), untreated EDTA-prepared virus at about 1 mg./ml. (B), a sample of the same virus preparation after heating. (C), Bentonite-prepared virus after heating. Before treatment this material appeared as in (A). (D), EDTA-prepared virus heated in the presence of about 1 mg./ml. sodium bentonite. Photographs taken approx. 17 min. after reaching a centrifugation speed of 35,600 rev./min. (VT), 'top' component; (VB), 'bottom' component; (R), 28 S component. Centrifugation was carried out using two cells in a rotor and in (B) the meniscus present in the accompanying cell is visible (M).

Fig. 2. Ultraviolet photographs taken at 4 min. intervals after reaching a centrifugation speed of 35,600 rev./min. (A), untreated bentonite-prepared virus; (B), a sample of the same preparation after heating. The optical density of the solutions at 260 m\(\mu\) was about one.
Fig. 1

Fig. 2

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