Cross-linking of RNA Induced by Ultraviolet Irradiation of Particles of Raspberry Ringspot Virus

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Raspberry ringspot virus (R/1: 2.4/43 + 1.4/30 (or 2 × 1.4/46): S/S: S/Ne, nepovirus group) sediments as three major components (T, M and B) with sedimentation coefficients of 52, 92 and 130 S, respectively (Mayo, Murant & Harrison, 1971). We reported (Murant et al. 1972) that particles of M component yielded one type of RNA molecule, of mol. wt. 1.4 × 10⁶ (RNA-2), whereas B component yielded both RNA-2 and RNA of mol. wt. 2.4 × 10⁶ (RNA-1). We suggested that some particles of B component contain one RNA-1 molecule and others contain two RNA-2 molecules. Confirmation of this suggestion comes from the finding, which we now report, that dimers of RNA-2 are formed when purified virus is irradiated with u.v. light.

The culture of raspberry ringspot virus, and the methods by which it was purified and assayed for infectivity, were as described by Murant et al. (1972). RNA was isolated from virus preparations using the pronase/SDS procedure and heated at 60 °C for 15 min in electrophoresis buffer containing 8 M-urea + 0.2 % SDS, before electrophoresis in 2.2 % polyacrylamide gels (Murant et al. 1972). Samples (1 or 2 ml, E₂₆₀ = 1 to 2) of virus, in 0.02 to 0.05 M-phosphate buffer, or of RNA, in either 0.15 M-sodium chloride + 0.015 M-sodium citrate (SSC) or 0.15 M-sodium acetate + 0.5 % SDS, pH 6.0 (acetate-SDS), were irradiated with u.v. light, without stirring, in 5 cm diameter dishes using a low-pressure mercury discharge lamp (Hanovia Ltd.). At least 95 % of the radiation was at 254 nm and the radiation intensity at 20 cm (the distance between lamp and dish) was 720 μW/cm², measured by potassium ferrioxalate actinometry (Hatchard & Parker, 1956).

Irradiation for 10 s decreased the infectivity of virus about 50-fold, and virus irradiated for more than 1 min was virtually non-infective. When virus was irradiated for 4 min and the RNA analysed in acrylamide gels, the relative size of the RNA-2 peak was decreased (Fig. 1b) by comparison with that from unirradiated virus (Fig. 1a). Extra material (photoproduct) was found in the RNA-1 region of the gel, and this had a slightly lower mobility (equivalent to mol. wt. of 2.4 to 2.8 × 10⁶) than untreated RNA-1. In some gels the two were partially resolved (Fig. 1c). We therefore suggest that the photoproduct is a dimer formed by a cross-linking reaction between RNA-2 molecules. Further evidence for this suggestion was obtained when RNA preparations from irradiated or unirradiated virus were compared by sedimentation at 36000 rev/min using a Beckman type II band forming centrepiece in an analytical ultracentrifuge (Bauer & Vinograd, 1971). Two bands of RNA were detected using a sedimentation solvent of 1 M-sodium chloride + 0.05 M-disodium hydrogen phosphate, and RNA from irradiated virus contained relatively more of the faster sedimenting band, and less of the slower sedimenting band, than did RNA from unirradiated virus.

The electrophoretic mobility and amount of the RNA-2 dimer were not affected when RNA extracted from irradiated virus was treated with phenol, or with 8 M-urea at 60 °C, treatments which should break non-covalent bonds between nucleic acid molecules or between protein and nucleic acid. Because pronase was used to prepare the RNA, and gels containing RNA from virus irradiated with u.v. light did not stain with Coomassie blue, the RNA preparations did not contain substantial amounts of protein, although we cannot
Fig. 1. Electrophoresis of approximately 40 μg RNA from unfractionated raspberry ringspot virus in 2.2% acrylamide gels in 0.02 M-tris-phosphate + 0.002 M-EDTA + 0.2% SDS, pH 7.8, 7 V/cm, 6 mA/gel in 6 mm diameter gels, for 250 min (a, b) or 200 min (c). (a) RNA from control virus. (b) RNA from u.v.-irradiated virus (4 min). (c) As (b), but in this gel RNA-1 is partially resolved from RNA-2 dimer. Electrophoretic migration is from left to right.

exclude the possibility that they contained some covalently bound, pronase-resistant peptide material not detected by staining. The band of RNA-2 dimer in gels was rather broader than that of RNA-1, possibly because a range of conformational forms existed, or because the linkage occurred at different sites in the RNA-2 molecules, resulting in a range of molecular configurations. No consistent differences in u.v. absorption spectra were detected between irradiated and unirradiated virus, or between their respective RNAs.

In further experiments on dimer formation, preparations of M or B components, obtained by two cycles of sucrose density gradient sedimentation (Murant et al. 1972), were irradiated and their RNAs examined by gel electrophoresis. The electrophoretic mobility and the yield of RNA-2 from M component were unchanged by irradiation for 4 min, although the peak was slightly broadened (Fig. 2a, b). In contrast, irradiation of B component for only 30 s (Fig. 2d) caused a change in the mobility of some RNA as compared with the unirradiated control (Fig. 2c), and longer treatments further increased the amount of RNA-1 + RNA-2 dimer and decreased the amount of RNA-2 (Fig. 2e, f). Using the areas under the absorbance peaks as an approximate measure of the amount of RNA in the peaks (Fig. 2c to f), the ratios of the amount of RNA-1 + RNA-2 dimer to the amount of RNA-2 are 0.4 (no irradiation), 0.6 (30 s), 1.2 (1 min) and 3.5 (4 min). RNA-2 dimers were
Fig. 2. Electrophoresis of RNA obtained from u.v.-irradiated M and B components of raspberry ringspot virus. Electrophoresis in 2.2% acrylamide gels as described in Fig. 1, for 190 min. (a) RNA from about 200 μg unirradiated M component. (b) As (a), RNA extracted after u.v. irradiation for 4 min. (c) RNA from about 150 μg unirradiated B component. (d) As (c), RNA extracted after u.v. irradiation for 30 s. (e) As (c), RNA extracted after u.v. irradiation for 1 min. (f) As (c), RNA extracted after u.v. irradiation for 4 min. Electrophoretic migration is from left to right.

not formed by irradiating RNA from unfractionated virus for up to 16 min in either SSC or acetate-SDS. When RNA preparations containing RNA-2 dimer were irradiated for up to 16 min in either SSC or acetate-SDS, dimers were not converted to monomers, and it is therefore unlikely that dimers were formed and then immediately photoreversed when unirradiated RNA was irradiated. Thus dimers were produced only by irradiating RNA-2 when it was within particles of B component, and these results therefore support the suggestion by Murant et al. (1972) that there are two types of particle in B component, one containing one molecule of RNA-1, the other containing two molecules of RNA-2.
Fig. 3. Schlieren diagrams of a preparation of raspberry ringspot virus centrifuged to equilibrium in CsCl solution of initial density 1.516 g/cm³ in 0.005 M-phosphate buffer, pH 7. Schlieren angle 60°. T particles are at the meniscus and M particles have banded close to the meniscus. (a) Kept at 2 °C at all stages and centrifuged at 44,000 rev/min for 18 h; note the two major classes of B particles. (b) As (a) but subsequently stored for 3 days at 5 to 15 °C, then 5 h at 27 °C, before centrifuging at 44,000 rev/min and 10 °C for 18 h. Note the single band of somewhat aggregated B particles.

In earlier work, we failed to detect two kinds of particle in B component by equilibrium sedimentation in CsCl at 25 °C (Murant et al. 1972). However, we now find that when the CsCl solution containing raspberry ringspot virus is kept close to 2 °C at all stages, B component separates into two classes of particles (Fig. 3a), of estimated density 1.51 g/cm³ and 1.53 g/cm³. The preparation that gave Fig. 3a was subsequently stored in the cell at 5 to 15 °C for 3 days and then at 27 °C for 5 h; when it was centrifuged again, the B component formed a single zone of somewhat aggregated particles of density 1.54 g/cm³ (Fig. 3b). Particles of M component, however, seemed to aggregate less.

We estimate that the dose of u.v. radiation needed to dimerize half the RNA-2 in B component is of the order of 100 times that required to inactivate half the virus infectivity. Thus the event that leads to dimerization is relatively rare, or the two RNA-2 molecules that occur in one type of B component particle are favourably orientated at relatively few sites. We know of no published information showing that u.v. radiation induces cross-linking of RNA within virus particles, but it is known to induce cross-links between DNA molecules and to cause covalent binding between DNA and protein (Rahn, 1972). Our results do not suggest that protein is involved in the cross-linking of RNA-2 in raspberry ringspot virus particles, and a similar conclusion was reached by Francke & Ray (1972), who studied the effect of u.v. irradiation on the single-stranded circular DNA in the bacteriophages ØX 174 and M 13. They found that, following a dose of u.v. radiation sufficient to inactivate 99.85 % of the infectivity of ØX 174, about 38 % of the DNA molecules were internally cross-linked. The photo-chemical reactions responsible for this effect may be similar to those that result in cross-links between RNA-2 molecules of raspberry ringspot virus.

We have found (Murant et al. 1972; Harrison, Murant & Mayo, 1972) that preparations of RNA-1 are contaminated with RNA-2, possibly occurring as dimers, and that much of this contamination can be eliminated by heating RNA in 8M-urea. The photoprodust we describe may represent such dimers, made stable to urea by u.v. irradiation. Whether or not this is so, u.v. irradiation may prove useful as a general method for determining which RNA molecules occur together within virus particles.
Short communications

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