

Deoxyribonuclease Digestion of the Nucleic Acid from Carnation Etched Ring Virus

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Accepted for publication 5 October 1971.

ABSTRACT

Carnation etched ring virus was purified from infected *Silene armeria* by differential centrifugation and sucrose density-gradient centrifugation. The purified preparation revealed a typical ultraviolet absorption spectrum for nucleoprotein, and consisted of infective particles about

42 nm in diameter. The nucleic acid isolated from the purified virus was digested completely with deoxyribonuclease, but not digested with ribonuclease.

Phytopathology 62:810-811.

In a previous paper (1), it was reported that particle morphology and intracellular appearance of carnation etched ring virus (CERV) closely resembled those of cauliflower mosaic virus (CAMV) (2), and thymidine-³H was incorporated preferably into CERV. This paper deals with further study of the nucleic acid isolated from CERV.

Lower leaves of *Silene armeria* L. were inoculated mechanically with crude sap of *Dianthus caryophyllus* L. infected with CERV. About 1 month after inoculation, systemically infected young leaves

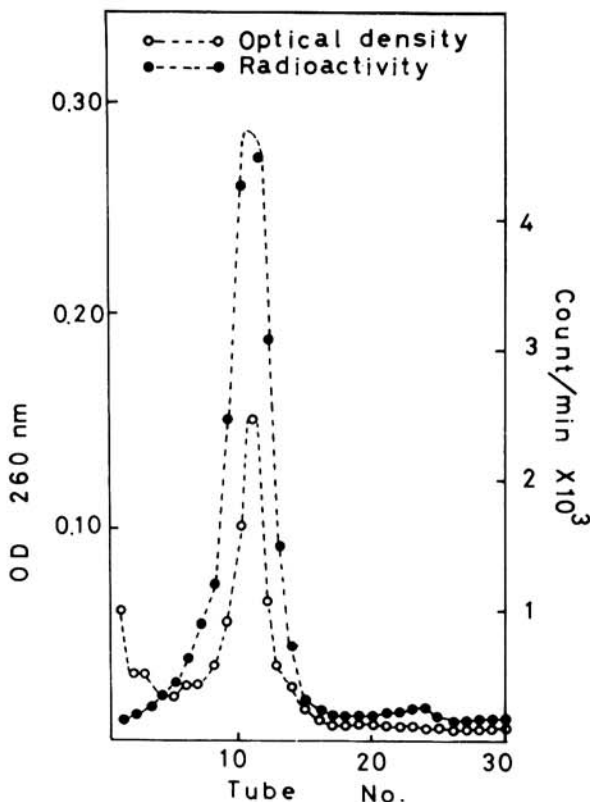


Fig. 1. The optical density profile and radioactivity profile of centrifuged sucrose density-gradient containing thymidine-³H-labeled carnation etched ring virus. Sedimentation from right to left, with the smaller numbers representing tubes from the bottom of the gradient.

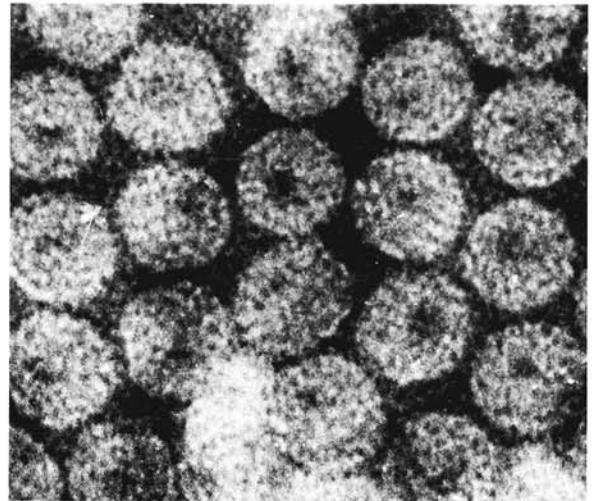


Fig. 2. Electron micrograph of negatively stained carnation etched ring virus particles ($\times 330,000$).

were used for thymidine-³H uptake as described previously (1). Techniques of purification and sucrose density-gradient centrifugation of the labeled leaves were those described previously (1).

The optical density profile and radioactivity profile of each fraction after sucrose density-gradient centrifugation are shown in Fig. 1. The radioactive and optical density peaks appeared at tube No. 11 (Fig. 1). Since electron microscopy of this fraction, stained negatively with 2% potassium phosphotungstate solution, revealed spherical particles about 42 nm in diam (Fig. 2), it was clear that this fraction consisted of CERV particles, and thymidine-³H was specifically incorporated into CERV particles.

Enzymatic hydrolysis of the nucleic acid isolated from thymidine-³H-labeled CERV was attempted with ribonuclease and deoxyribonuclease. The radioactive fractions (tube No. 10 to 13 in Fig. 1) were collected and dialyzed against 0.01 M phosphate buffer (pH 7.5) overnight. One ml of 0.2% pronase and 0.2 ml of 0.1 M EDTA were added to 6 ml of ³H-labeled CERV solution. After incubation at 37 C for 4 hr, 0.8 ml of 5% sodium dodecyl sulfate (SDS) was added to the solution. After incubation at 37 C

for 2 hr, an equal volume of water-saturated phenol was added to the solution. After a gentle swirling for 20 min, 1 mg of DNA (calf thymus) as a carrier was dissolved in the aqueous phase extracted from the solution. The nucleic acid was precipitated with two volumes of ethyl alcohol at -20°C overnight, sedimented by low-speed centrifugation, dissolved in 0.01 M Tris[tris (hydroxymethyl) amino methane]-HCl buffer (pH 7.8) and dialyzed against the same buffer overnight. For digestion with ribonuclease, 1 ml of the nucleic acid solution was incubated for 30 min at 37°C with pancreatic ribonuclease ($2\ \mu\text{g}/\text{ml}$) in the presence of 0.02 M EDTA. For digestion with deoxyribonuclease, 1 ml of the solution was incubated for 30 min at 37°C with pancreatic deoxyribonuclease ($2\ \mu\text{g}/\text{ml}$) in the presence of 0.002 M MgCl_2 . The control (no enzymes) was also left for 30 min at 37°C . These three samples were layered onto separate sucrose density-gradients (4 to 20%) made with 0.01 M Tris buffer (pH 7.8) and centrifuged at 24,000 rpm at 4°C for 24 hr. After centrifugation, each gradient was divided into 35 fractions from the bottom of the gradient tube. The radioactivity of each fraction was assayed in a liquid-scintillation counter.

The control exhibited a single radioactive peak at tube No. 14 to 18 (Fig. 3-A). The sample treated with ribonuclease showed a radioactivity profile similar to that of the control (Fig. 3-B). The sample treated with deoxyribonuclease revealed no radioactivity peak at tube No. 14 to 18, and the radioactivity removed near the meniscus (Fig. 3-C). Thus, it is concluded that the nucleic acid isolated from CERV was susceptible to deoxyribonuclease but resistant to ribonuclease. These results suggest that the nucleic acid in CERV is DNA. Furthermore, the present results were supported in situ in the cells by electron microscopic radioautography (Fig. 4) of

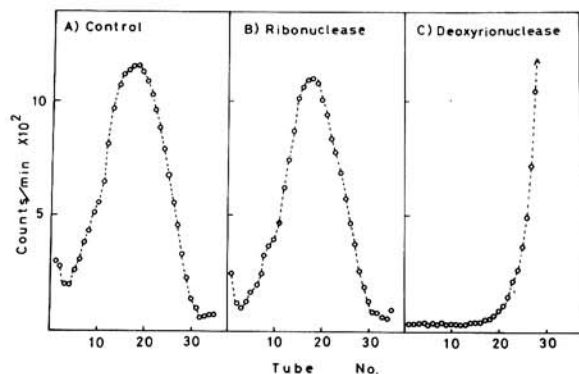


Fig. 3. Radioactivity profile of centrifuged sucrose density-gradient containing nucleic acid isolated from thymidine- ^3H -labeled carnation etched ring virus. A) Control. B) Treated with ribonuclease. C) Treated with deoxyribonuclease.

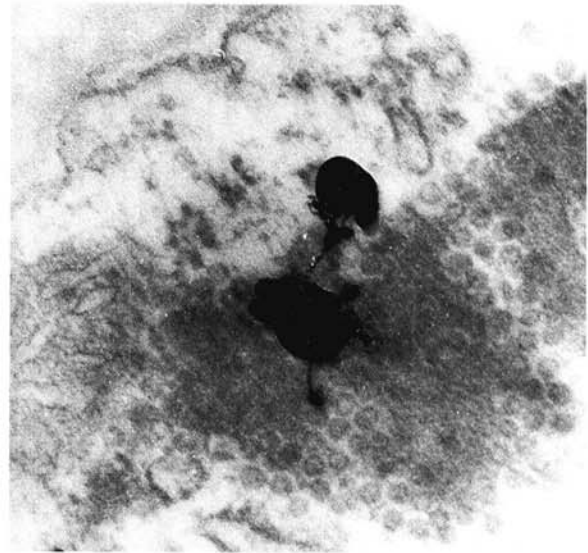


Fig. 4. Electron-microscopic radioautograph of thymidine- ^3H labeled X-body associated with carnation etched ring virus infection ($\times 63,000$).

CERV-infected *D. caryophyllus* leaves floated on thymidine- ^3H solution (0.2 mc/ml) for 48 hr, because most developed silver grains were observed over the X-bodies bearing CERV particles as well as those of CAMV (4). Shepherd et al. (5, 6) reported that DNA isolated from CAMV was double-stranded and was biologically active. Although detailed properties of DNA isolated from CERV are yet unknown, it is possible that DNA in CERV has similar properties to that in CAMV, because CERV is serologically related to CAMV (3).

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