Carnation Etched Ring Virus: Purification, Stability of Inclusions, and Properties of the Nucleic Acid

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ABSTRACT

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Carnation etched ring virus (CERV) was partially purified from mechanically inoculated fresh leaf tissue of *Saponaria vaccaria* 'Pink Beauty.' The yield of CERV from crude sap leaf extracts or from partially purified and concentrated CERV inclusion bodies was increased by treatment of extracts with butanol, urea, and Triton X-100 compared to butanol treatment alone. However, many inclusion bodies were not degraded by the treatment. Carnation etched ring virus nucleic acid (CERV-NA) occurs as linear forms of heterogeneous length as well as circular molecules. The molecular weight of the circular forms ranged from 4.21-4.31 $\times 10^6$ daltons. Highly twisted molecules without evidence of free ends also may be circular forms, but this could not be determined with certainty. The CERV-NA is completely hydrolyzed by DNase. After treatment with ethidium bromide, both CERV-NA and cauliflower mosaic virus NA bind to carbon-coated grids. In polyacrylamide-agarose gels the CERV-NA was resolved into three fluorescent bands after staining with ethidium bromide. The two slower-migrating components, A and B, consisted of highly twisted molecules that may be circular, a few separated linear forms, and tangled masses of molecules. The fast-moving component C consisted of linear molecules of heterogeneous length. No circular forms were detected in the C component.

Additional key words: electron microscopy, electrophoresis, cytoplasmic inclusions.

Carnation etched ring virus (CERV) is a member of the cauliflower mosaic virus group. The virus contains DNA (2, 7) and forms cytoplasmic inclusion bodies characteristic of the caulimoviruses (7). It has been purified from *Dianthus caryophyllus* (1), *Silene armeria* (2), and *Saponaria vaccaria* (7); however, the generally low yields and poor quality of CERV has precluded critical comparative studies of this virus with other caulimoviruses.

A new purification method for cauliflower mosaic virus (CauMV) was recently reported (5). The new method gives consistently higher yields than those previously reported (10, 14). The low yields obtained previously were attributed to the use of butanol in clarification (5). In the improved procedure, urea and Triton X-100 are incorporated in the crude sap extract or the crude sap is centrifuged at low speed and the resuspended pellets are treated with urea and Triton X-100. The authors speculated that the modified urea/Triton X-100 treatment released virions by degrading inclusion bodies that contain a high concentration of virus.

This paper describes an improved procedure for purification of CERV, the effects of various additives on the stability of CERV inclusion bodies, electron microscopy of CERV-NA, and electrophoresis of the nucleic acid in polyacrylamide-agarose gels.

MATERIALS AND METHODS

Virus source.—Carnation etched ring virus was maintained in *Saponaria vaccaria* 'Pink Beauty.' *Saponaria* also was used to assay the infectivity of CERV and CERV nucleic acid (CERV-NA) preparations. Cauliflower mosaic virus obtained from the American Type Culture Collection (ATCC PV-45) was maintained in and purified from mustard-spinach (*Brassica perviridis* Bailey 'Tendergreen').

Inoculated leaves of *S. vaccaria* showing red ring and line patterns, necrotic patches or concentric chlorotic rings and spots, and one or two pairs of systemically infected leaves showing curling symptoms were harvested 18-20 days after inoculation. Red ring and line patterns developed on leaves of some plants throughout the year in the greenhouse but were most prominent when temperatures were 22-28 C with light intensities of 15,000 lux. The highest yields of virus were obtained from plants grown in that environment and infectivity assays were performed under those conditions. Chlorotic rings and spots were most abundant on plants grown in the greenhouse in summer with temperatures exceeding 32 C and light intensities of 30,000 lux.

Virus purification.—Infected tissue was extracted immediately after harvest, without freezing. In most experiments, 150-175 g of tissue were homogenized in four-to-five volumes of 0.1 M Na_2HPO_4-KH_2PO_4, pH 7.2 (PO_4 buffer). The addition of the reducing agent $NaHSO_3$

did not affect infectivity of crude sap or yield of virus. Butanol was added to a final concentration of 8% and stirred for 2 hr at 4 C. The virus was further concentrated and purified by differential- and sucrose density gradient centrifugation. In comparative extractions, urea (6% w/v) and Triton X-100 (2.5%, v/v) were added to the extract after the initial butanol treatment and the mixture was stirred for 18-19 hr at 4 C (5). The extract was centrifuged at 5,000 g for 15 min and virus was concentrated from the supernatant fraction by centrifuging at 29,000 rpm for 2.5 hr in a Spinco No. 30 rotor. High-speed centrifugation pellets were resuspended in 0.01 M PO_4 buffer. Resuspended pellets, in 1-2 ml of buffer, were centrifuged in linear 10-40% (w/v) sucrose density gradients in 0.01 M PO_4 buffer, pH 7.2, for 2.5 hr at 24,000 rpm in a precooled Spinco SW 25.1 rotor at 5 C. Centrifuged sucrose density gradients (SDG's) were fractionated and analyzed with an ISCO Model D density gradient fractionator and UV-analyzer (Instrumentation Specialties Co., Lincoln, NE 68504). Virus yield was assessed by evaluating the shape of UV-absorbance profiles of centrifuged SDG's and the area under UV-absorbing, virus-containing peaks. Virus concentrations were calculated using a specific extinction coefficient of 7 $cm^2 mg^{-1}$ (uncorrected for light scattering) (12).

Inclusion bodies.—Inclusion bodies were extracted from 30 g of *S. vaccaria* leaves (inoculated, systemically infected, and showing leaf curl) by homogenization in four-to-five volumes of 0.1 M PO_4 buffer, pH 7.4, or in distilled water. Triton X-100 was added to the homogenate to a final concentration of 5% (v/v) and the mixture was stirred for 2 hr at 4 C. The extract was sieved successively through 417-μm, 149-μm, 105-μm, and 74-μm (mesh) screens and the material passed through the screens was concentrated by centrifugation at 3,000 g for 10 min. The pellets were resuspended in 5 ml of 0.1 M PO_4 buffer or in distilled water and treated with butanol, urea, and Triton X-100 to determine the effect of these additives on release of virions from the inclusion bodies.
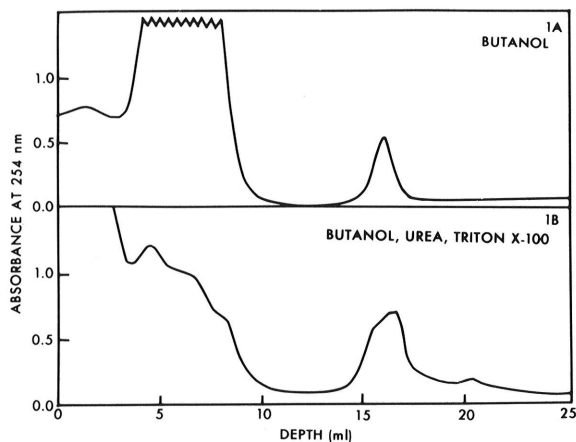
One ml of the extract containing 8% butanol, 6% urea, and 2.5% Triton X-100 was stirred for 18 hr at 4 C. Then the treated sample and an untreated control, the latter stirred with only 1 ml of distilled water, were centrifuged at 3,000 g for 10 min. A 1.2-ml sample of the supernatant fraction was layered on a sucrose density gradient as described above for purification of the virus and analyzed as described above. The low-speed pellets from the clarified treated and untreated extracts were resuspended in 0.01 M PO_4 buffer or in distilled water, stained with 1% phloxine, and examined in the light microscope.

Nucleic acid extraction.—Nucleic acid (NA) was extracted from CERV taken from the major UV-absorbing zone in sucrose density gradients essentially as described by Shepherd et al. (14). The virus was treated successively with 0.5-0.6% nuclease-free Pronase (predigested for 2 hr at 37 C) or 1.0 mg Pronase per milligram of virus and 1% SDS at 38-39 C for 1 hr each, followed by extraction with buffer-saturated phenol. The NA was precipitated with two volumes of cold 95% ethanol and a few drops of 3 M sodium acetate (pH 5.1) per milliliter. The NA was collected by low-speed centrifugation and resuspended in 0.01 M PO_4 buffer or 0.01 M Tris-HCl, 0.001 M Na_2EDTA_2, pH 7.5 (TE buffer). Nucleic acid concentrations were determined spectrophotometrically using an extinction coefficient of 20 $cm^2 \cdot mg^{-1}$ at A_{260} nm and a 1-cm light path (12). Nucleic acid was also extracted from cauliflower mosaic virus by this method following purification of the virus with butanol, urea, and Triton X-100 (Civerolo and Lawson, *unpublished*).

Electron microscopy.—The CERV-containing zone and fractions below the major zone were collected from centrifuged density gradients and dialyzed in 0.01 M PO_4 buffer, pH 7.2. The samples were dropped on a Formvar-coated grid and stained with an equal volume of 2% PTA adjusted to pH 7.0 with KOH.

Carnation etched ring virus NA in TE buffer containing about 0.15 A_{260}/ml was diluted by mixing 0.1

Fig. 1-(**A, B**). Ultraviolet absorption profile of a centrifuged sucrose density gradient containing carnation etched ring virus from a tissue extract treated with **A**) butanol only and **B**) butanol, urea, and Triton X-100. Sedimentation is from left to right.
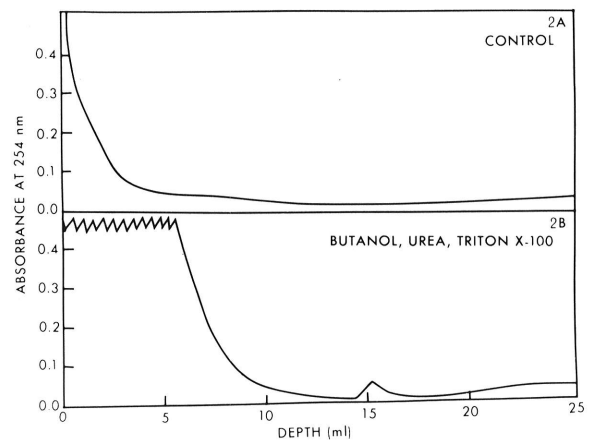

Fig. 2-(**A, B**). Ultraviolet absorption profile of the supernatant liquid from a carnation etched ring virus inclusion extract that was stirred for 18 hr and clarified by low-speed centrifugation before density gradient centrifugation. **A**) Inclusion extract treated only with distilled water and **B**) treated with butanol, urea, and Triton X-100. Sedimentation is from left to right.

ml of sample with 0.9 ml of 1 M ammonium acetate, pH 7.2, and 0.01% cytochrome C (Type III, Sigma Chemical Co., St. Louis, MO 63178) and spread on a hypophase of 0.15 M ammonium acetate, pH 7.2, similar to the method previously described (6). The grids were rotary shadowed at an angle of 8 degrees with 80-20% Pt-Pd. Length of the molecules was measured with a map measurer from images magnified $\times$ 79,500 and compared to 312-nm and 500-nm (diameter) polystyrene latex spheres.

Nuclease treatments.—Bovine pancreatic DNase I (Calbiochem, Los Angeles, CA 90063) was dissolved in 0.01 M PO_4 buffer, pH 7.0, at 100 μg/ml. Carnation etched ring virus NA was incubated with dilutions of stock DNase with 10^{-3}M $MgCl_2$ and assayed on *S. vaccaria* at 0 time and after 30 min incubation at 37 C. Ribonuclease (Calbiochem) at 100 μg/ml in 0.01 M PO_4 buffer, pH 7.0, was boiled for 10 min to inactivate any contaminating DNase. Diluted enzyme was mixed with the NA and incubated with control NA samples without enzyme for 30 min at 37 C and assayed on *S. vaccaria*.

The DNA molecules were directly adsorbed to carbon-coated grids following reaction with ethidium bromide (15). To test the sensitivity of cauliflower mosaic virus and carnation etched ring virus nucleic acids to DNase, 10 μg/ml DNase was added to 5 μg of each nucleic acid preparation. The mixtures were incubated 30 min, mixed with 100 μg/ml ethidium bromide, dropped on Parafilm, and picked up on carbon grids. The grids were rotary shadowed with Pt-Pd.

Polyacrylamide-agarose gel electrophoresis.—Composite gels of 1.7 to 2.2% polyacrylamide and 0.5% agarose were prepared in 6 mm (diameter) $\times$ 10-14 cm (long) cylindrical tubes. Samples (50-100 μliters) containing 1-4 μg of CERV-or CauMV-NA were electrophoresed at 2.5-3.3 V/cm for 12 hr at room temperature. Electrophoresis buffer was 0.05 M Tris, 0.02 M Na-acetate, 0.002 M EDTA, 0.018 M NaCl (pH 8.0). The bands were viewed under short-wavelength UV light after the gels were soaked for 0.5-12 hr in 1 μg/ml of ethidium bromide.

The electrophoretic components of CERV-NA were recovered from the gels for electron microscopy. Slices containing the fluorescent bands were cut out of the gels and the nucleic acid in the band was eluted electrophoretically into dialysis bags (9). The samples were spread by the protein monolayer procedure (6) and the conformation of the strands was examined in the electron microscope and assayed on *S. vaccaria* for infectivity.

RESULTS

Virus purification.—Inoculated *S. vaccaria* leaves showing symptoms and systemically infected leaves with distinct leaf curl symptoms yielded 50-400 μg of CERV per 100 g of fresh tissue treated with butanol only or frozen tissue treated with butanol, urea, and Triton X-100. Butanol treatment of fresh tissue extracts produced a virus-containing zone free of contaminating, UV-absorbing material in a sucrose density gradient column (Fig. 1-A). From equivalent amounts of tissue, the combination of 8% butanol, 6% urea, and 2.5% Triton X-100 sometimes yielded more virus in extracts from fresh

tissue than butanol alone (Fig. 1-B). The major UV-absorbing, virus-containing zone from the butanol, urea, and Triton X-100-treated extracts was opalescent, but usually contained a greenish light-scattering material. Increased absorbance at 254 nm in the virus zone resulted from a broader UV-absorbing peak as well as an increase in the height of the peak. In a few experiments, there was a shoulder on the trailing edge of the major peak (Fig. 1-B). Samples from the trailing edge were mostly separate virions in the electron microscope. In the leading portion of the peak there were many single dispersed virions and aggregates with varying numbers of particles. In most experiments there was no shoulder on the trailing edge of the major peak from extracts treated with butanol, urea, and Triton X-100. In this peak some clusters of virions were intermixed with the population of mostly separate particles.

A second small peak below the major UV-absorbing zone occurred in some preparations treated either with butanol only, or butanol, urea, and Triton X-100. There was no evidence of regular aggregates of dimers or trimers in the sample from this peak. A few virions were present as separate particles but most of the particles were in clusters.

Infectivity.—Infectivity in extracts from frozen *S. vaccaria* leaves was associated with the major UV-absorbing peak, but was not confined to this peak in bioassays on *S. vaccaria* plants (Lawson and Civerolo, *unpublished*). Fractions below the major UV-absorbing zone in the denser portion of the gradient also were infectious. A similar pattern of infectivity distribution was obtained whether fresh tissue extracts were treated with butanol only, or with butanol, urea, and Triton X-100. Infectivity consistently was present in the fractions below the major zone although the second small UV-absorbing peak was not always present. Based on the appearance of the UV profile and the relatively low concentration of virus recovered from the major zone, we concluded that these results cannot be explained by an overloading of the density gradient.

Inclusion bodies.—In the electron microscope a few separated virions were seen in PTA-stained preparations of the nonfractionated inclusion extract in water and in the extract treated with 0.1 M PO_4 buffer that were layered on the sucrose density gradients. Some diffuse nonstaining material with associated virions also was in the sample. Following centrifugation, no UV-absorbing zone was in the sucrose gradients layered with either the water or PO_4 extract (Fig. 2-A).

Unfractionated extracts of inclusion bodies in water or in 0.1 M PO_4 buffer treated with 8% butanol, 6% urea, and 2.5% Triton X-100 for 18 hr and then stained with neutral PTA, contained many separated virions and structures that were apparently inclusion fragments. The density of most of these bodies precluded observation of ultrastructure, but occasionally clusters of virions with some overlapping particles were associated with the fragments (Fig. 3-a). The virions measured only about 36 nm in diameter but the outline was poorly defined by the surrounding material. A distinct UV-absorbing zone was in the gradient containing the water or PO_4 extracts that were treated with butanol, urea, and Triton X-100 (Fig. 2-B). Virions in this zone were separated or in clusters and
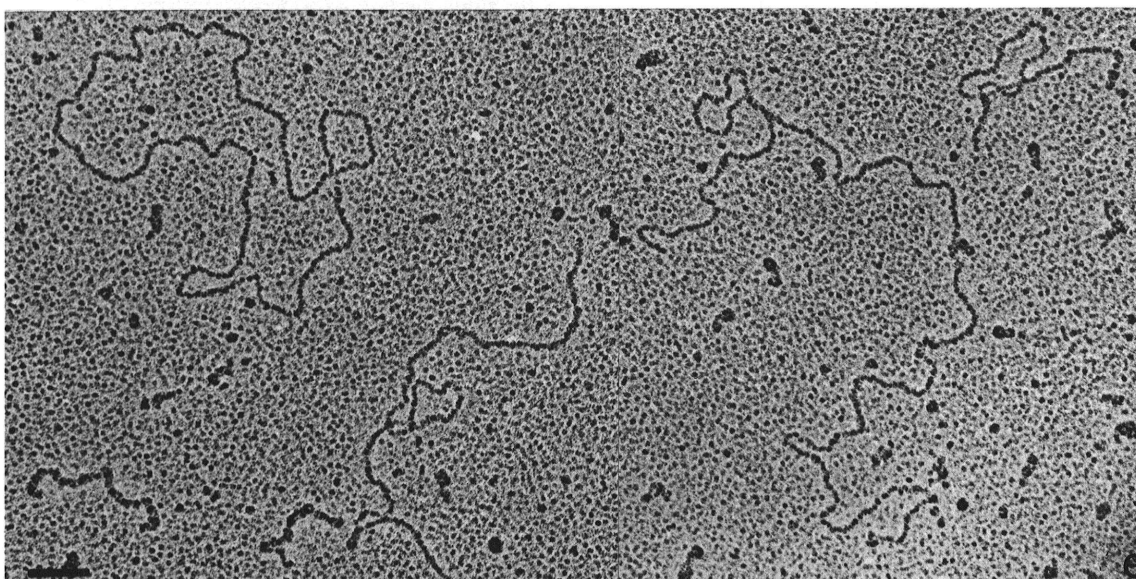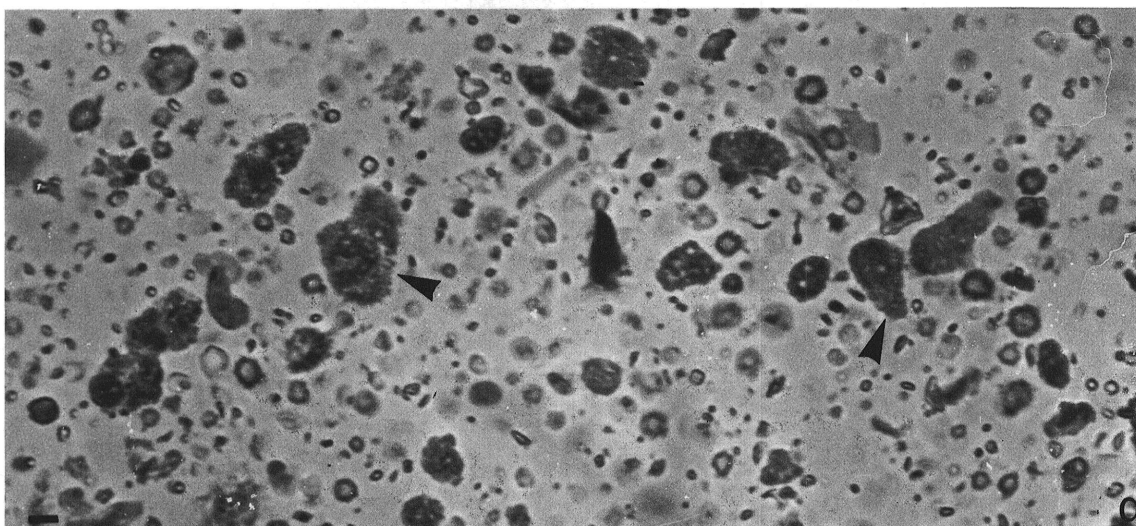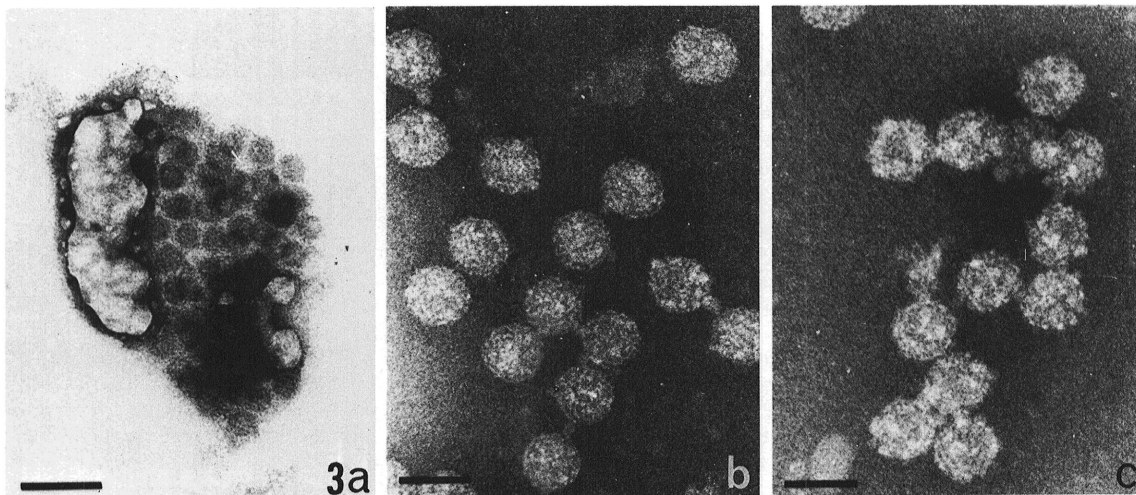
measured 42-44 nm. Between virions in some clusters, there appeared to be material that may have caused the aggregation (Fig. 3-b). In other clusters, the entire surface of the particle apparently was coated with material (Fig. 3-c).

Many inclusions in the resuspended pellets of the control samples extracted in 0.1 M PO_4 or water were still intact and stained densely with 1% phloxine. Inclusions in the PO_4 or water extracts that were treated for 18 hr with butanol, urea, and Triton X-100 also stained densely (Fig. 3-d). Although some of the inclusions in the treated samples showed an irregular outline with possible fragmentation, the procedure failed to degrade many of the inclusions and release the virions.

Nucleic acid.—In most experiments, CERV-NA occurred predominantly as linear forms with only a few circular molecules without crossovers (Fig. 3-e). In one experiment, 45% of 168 linear molecules measured 0.12-0.37 μm long. Thirty percent of the molecules were 0.38-0.62 μm long, with some molecules measuring about 1.8-2.1 μm (Fig. 4-A). Only three circular molecules 2.15-2.25 μm with no crossovers could be measured. Assuming a molecular weight of $1.96 \times 10^6/\mu$m (6), the molecular weight of the circular molecules would correspond to 4.21×10^6 daltons. An additional 32 molecules were highly twisted and no free ends were observed. We assume that these molecules also were circular.

In a second experiment, nine circular forms ranging in length from 1.87-2.25 μm were measured in a population

of 129 molecules (Fig. 4-B). Twenty-one additional molecules were highly twisted and were probably circular. Thirty-eight percent of the measured molecules were 0.12-0.37 μm and 18% ranged from 0.38-0.62 μm.

In a third experiment, 66 out of 137 molecules were linear. There were 21 circular molecules with up to three crossover points and the remainder of nonlinear forms were highly twisted without free ends and also may have been circular forms.

The most prevalent lengths of the linear forms were 0.25 μm, 0.37 μm, and 0.5 μm. The molecular weights of these pieces correspond to 0.49×10^6, 0.72×10^6, and 0.98×10^6 daltons, respectively. With the exception of one molecule, with an estimated molecular weight of 4.15×10^6 daltons, all of the circular forms that could be measured in the three experiments ranged from 4.21 to 4.31×10^6 daltons. If the circular molecule is composed of short forms, the number of segments in a single circular molecule could range from about four to eight depending on whether the molecule is composed of all short segments or longer linear molecules combined with shorter strands.

Nuclease treatments.—The infectivity of CERV-NA is low. At concentrations of 5 and 15 μg/ml CERV-NA was infectious to *S. vaccaria* with 4 of 10 and 5 of 12 inoculated plants showing symptoms, respectively, in each of two experiments, and 2 of 10 plants inoculated with 15 μg NA showing disease symptoms in the third experiment. At concentrations of 5 and 15 μg/ml CERV-NA was not infectious after treatment with 10 μg/ml DNase and incubation for 30 min at 37 C. With 15 μg/ml of NA 2 of 10 of the plants were infected in the 0-time inoculation following the addition of 10 μg/ml DNase. Results of tests with RNase were inconclusive. In one experiment 3 of 10 plants inoculated with 15 μg/ml of NA were infected, but none of 10 plants was infected after treatment of 15 μg/ml of NA with 10 μg/ml RNase and only 1 of 10 inoculated plants was infected after treatment with 1 μg/ml of RNase.

A high concentration of DNA molecules covered the surface of the grids of control preparations of CauMV-NA and CERV-NA incubated with 100 μg/ml ethidium bromide in the absence of DNase (Fig. 5-a and 5-b). No molecules were on grids incubated with DNase and then mixed with 100 μg/ml ethidium bromide. The DNA-ethidium bromide complex is positively charged in a low-ionic-strength buffer and binds to the negatively charged carbon surface (15). Unfolding of the DNA molecule under these conditions on the carbon film is consistent with the known property of ethidium bromide to intercalate and unwind the DNA helix (11).

Polyacrylamide-agarose gel electrophoresis.—Preparations of CERV-NA and CauMV-NA contain three electrophoretic components which we
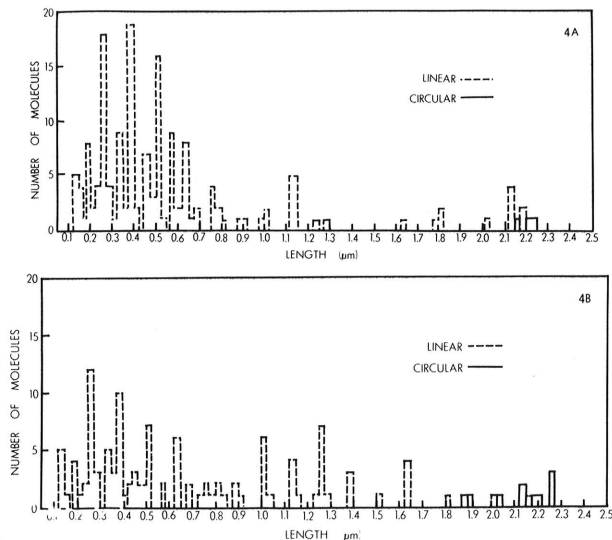
Fig. 4-(**A**, **B**). Length distribution of circular and linear forms of carnation etched ring virus nucleic acid from two experiments.

Fig. 3-(**a to e**). Carnation etched ring virus (CERV) inclusions and virions from treated inclusion extracts and CERV nucleic acid (NA). **a)** Inclusion fragment from a concentrate of CERV inclusions treated with butanol, urea, and Triton X-100 and stained with PTA. Bar = 100 nm. **b)** Clusters of CERV particles from the major UV-absorbing peak in a sucrose density gradient containing an inclusion extract and showing some material between the virions. Bar = 50 nm. **c)** Shows a higher concentration of material between the particles and coating the particle surface. Bar = 50 nm. **d)** Extracted CERV inclusion bodies (arrows) stirred for 18 hr with butanol, urea, and Triton X-100 and stained with 1% phloxine. Note the densely stained vacuolate inclusions. Bar = 5 μm. **e)** Circular molecule of CERV-NA without crossovers and some linear forms. Bar = 100 nm.

designate A, B, and C in increasing order of migration from the origin. The bands fluoresce in UV after staining with 1 μg/ml ethidium bromide. Initial experiments in 2.2% polyacrylamide-0.5% agarose gels consistently resolved component C that was in the same position in the gel with both CERV and CauMV-NA preparations but the relative amount of this component in CERV-NA preparations was greater and the band was more diffuse than in CauMV-NA preparations (7). Components A and B in CERV-NA preparations were not well resolved. In gels composed of 1.8% polyacrylamide-0.5% agarose, the A and B components as well as the diffuse band produced by the more rapid-migrating C component in CERV were clearly resolved (Fig. 6-a). The relative amount of CERV-NA-C was greater than from CauMV-NA-C and was more diffuse than CauMV-NA-C (7).

A gel slice containing both the A and B components from the 1.8% polyacrylamide-0.5% agarose gel contained highly twisted forms that were probably circular (Fig. 6-b). A few linear forms of heterogeneous length and a few masses of tangled strands also were in the A and B components. Component C contained only linear forms of virable length (Fig. 6-c). No infectivity resulted from inoculation of *S. vaccaria* with either the A and B, or C components from the gel.

DISCUSSION

Purification of CERV is accomplished with difficulty. We estimate that yields of virus from *Saponaria* leaf tissue are five to six times as great as the yield from *S. armeria* (2) based on the A_{260} of the major UV-absorbing virus-containing zone in a sucrose density gradient. The virus also has been purified from *D. caryophyllus* (1) but yields were low (7). In The Netherlands, H. Huttinga (*personal communication*) obtained 200-500 μg of CERV from 1 kg of carnation leaves.

Treatment of fresh infected tissue with a combination of butanol, urea, and Triton X-100 usually yielded more virus than butanol treatment alone. The yield of virus with the combined treatment was about twice the yield from the butanol treatment alone in some experiments. In other experiments, the yield from the combined treatment was increased about 20-30%. Electron microscope observation of fractions from the major UV-absorbing peak showed that many separated virus particles were in the fractions from both crude sap treated only with butanol and the sap treated with butanol, urea, and Triton X-100. However, fewer clusters or aggregates of virions were in the gradient fractions from the major UV-absorbing peak from the sap that received only butanol.

Treatment of isolated inclusions with butanol, urea, and Triton X-100 confirmed that these additives do release virions from the inclusions in sufficient quantity to be detected in a sucrose density gradient. Increased yields may result from degradation of the least stable inclusions in the population. Inclusions differ in the amount of matrix they contain and those inclusions and inclusion fragments with the least matrix may be the most susceptible to breakdown. Inclusions that have been partially purified may be the more stable forms in the population because the larger intact bodies and not the small fragments would be concentrated by low-speed centrifugation. Thus, the relatively low amount of virus released from the partially purified inclusion population would represent a release of particles from only the more resistant forms and would not account for the increased amount of virus released from the less-stable inclusions and inclusion fragments in the original crude sap preparation.

Dahlia mosaic virus (3) and CauMV (Civerolo and Lawson, *unpublished*) showed a small UV-absorbing zone below the major zone and infectivity was associated with this zone from CauMV preparations. Infectivity associated with fractions in CERV preparations below the major UV-absorbing zone may result from particle aggregates that do not separate upon dilution. Although the A_{260}/ml in this portion of the density gradient is low, the relative efficiency of the virion clusters in producing infection may be greater than the efficiency of separated particles. It is unlikely that the virions in this minor, rapid-sedimenting peak are nuclear virions aggregated with material from the nucleoplasm since nuclear associated virions have not been reported (13) with CauMV or DaMV. We cannot, however, preclude the possibility that the distribution of CERV infectivity below the major UV-absorbing zone is due to a multipartite genome.

Although we have been unable to demonstrate the presence of DNA in the matrix of CERV inclusion bodies
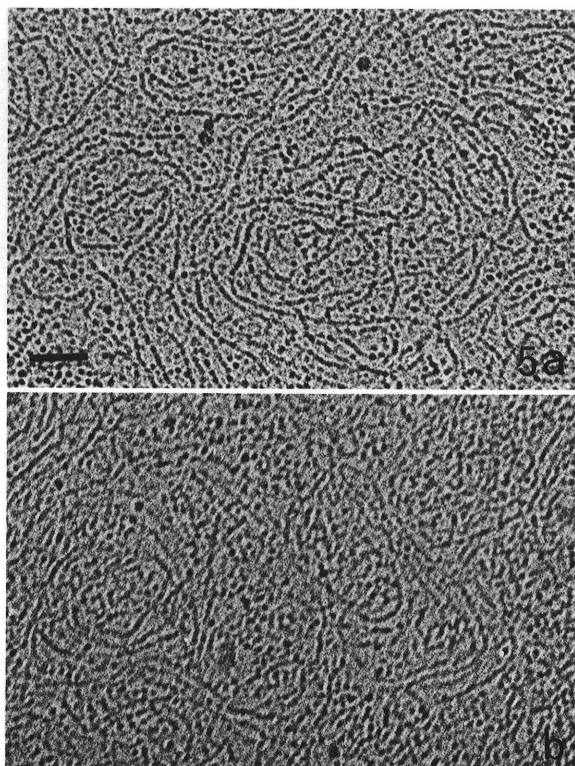

Fig. 5-(a, b). Cauliflower mosaic virus nucleic acid (CauMV-NA) and carnation etched ring virus nucleic acid (CERV-NA) treated with 100 μg/ml ethidium bromide, and adsorbed to carbon grids. a) CauMV-NA. b) CERV-NA. Note the high concentration of molecules attached to the grids. Bar = 100 nm.

by enzyme histochemistry (Lawson and Hearon, *unpublished*), Shepherd (13) hypothesized that the inclusion bodies are sites of both virus assembly and DNA replication.

Increased yields of CERV from infected *S. vaccaria* leaves compared to *S. armeria* and *D. caryophyllus* may result from the release of nuclear virions (8). There is no evidence that matrix material is associated with nuclear virions. Disruption of nuclei during the initial extraction may free many virions as separated particles. Alternately, the virions may be associated with chromatin or nucleoplasm. Clusters of virions associated with stranded material in the sucrose density gradient may be of nuclear origin.

Preparations of CERV-NA are distinguished from those of CauMV-NA by the occurrence of more linear than circular forms in many CERV-NA preparations. Nucleic acid extracted at the same time from both CERV and CauMV purified the same way showed this difference between CERV-NA and CauMV-NA in many different experiments over a 2-yr period. The percentage of circular and highly crossed CERV-NA molecules without free ends varied among experiments, but the calculated MW of 4.15-4.31×10^6 daltons was consistently obtained for
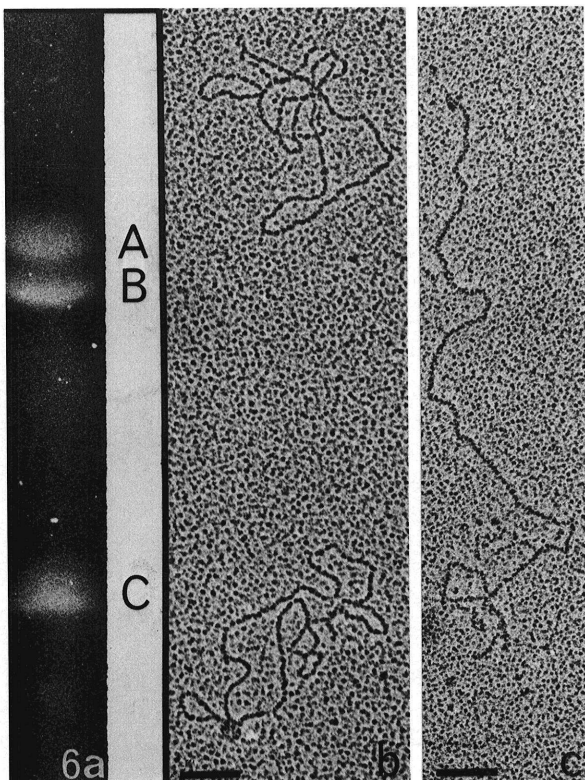

Fig. 6-(a to c). Electrophoresis of carnation etched ring virus nucleic acid (CERV-NA) in a composite 1.8% polyacrylamide-0.5% agarose gel and electron micrographs of molecules recovered from the gel. **a)** Gel showing diffuse band of component A (nearest top of gel) separated from B and the most rapidly migrating diffuse C component (bottom of gel). **b)** Highly twisted CERV-NA molecules recovered from components A and B. Bar = 100 nm. **c)** Linear form of CERV-NA recovered from component C. Bar = 100 nm.

the circular forms. The linear forms may be fragments of the circular forms or possibly represent a difference in the structure of NA associated with the nuclear virions. Denaturation of CauMV-NA with alkali yields single-strand pieces of DNA with an average molecular weight of 4×10^5 daltons (4). The heterogeneity in length of short linear molecules of CERV-NA did not allow us to determine if the genome is composed of specific segments.

The sensitivity of CERV-NA to DNase reported here confirms previous reports that CERV is a DNA-containing virus (2). Attachment of a high concentration of CauMV-NA and CERV-NA molecules to carbon-coated grids following reaction with ethidium bromide is consistent with the hypothesis that CERV-NA, like CauMV-NA, also is double-stranded.

The similarity between CERV-NA and CauMV-NA is indicated by the similar electrophoretic migration patterns of the nucleic acids in polyacrylamide-agarose gels. The slowest-migrating A and B bands were not consistently observed with CERV-NA in 2.2% polyacrylamide-0.5% agarose gels but a 1.8% polyacrylamide-0.5% agarose gel gives a reproducible pattern that appears very similar to the pattern produced by CauMV-NA. The highly twisted, apparently circular forms recovered from the A and B bands of CERV-NA in the 1.8% polyacrylamide-0.5% agarose gel is consistent with the observation of similar highly twisted forms in the A component of CauMV-NA (Civerolo and Lawson, *unpublished*) and the less highly twisted circular forms from the B component of CauMV-NA. The C component of CERV-NA contains only linear forms and the more diffuse fluorescence associated with this band is consistent with the observation that this fraction contains a more heterogeneous population of linear forms than CauMV-NA. Failure to demonstrate infectivity associated with gel components A and B or C may be explained by the limited recovery of low concentrations of NA from the gels and the inability to infect a high percentage of *S. vaccaria* with the nonfractionated NA. Only about one-tenth to one-half the amount of NA used in infectivity assays of the nonfractionated preparations was layered on the gels and a maximum of 40% of the inoculated plants were infected with the nonfractionated preparations.

Further work must be done to determine if there is a native population of short CERV-NA strands intrinsic to the virion or if the short linear forms result only from degradation of the larger molecules.

LITERATURE CITED

1. FUJISAWA, I., M. RUBIO-HUERTOS, and C. MATSUI. 1971. Incorporation of thymidine-^{3}H into carnation etched ring virus. Phytopathology 61:681-684.
2. FUJISAWA, I., M. RUBIO-HUERTOS, and C. MATSUI. 1972. Deoxyribonuclease digestion of the nucleic acid from carnation etched ring virus. Phytopathology 62:810-811.
3. FUJISAWA, I., M. RUBIO-HUERTOS, and C. MATSUI. 1974. Deoxyribonucleic acid in dahlia mosaic virus. Phytopathology 64:287-290.
4. HULL, R., and R. J. SHEPHERD. 1975. The proteins and nucleic acid of cauliflower mosaic virus. Page 92 *in* Proc. 3rd Int. Congr. Virol.; Abstracts. 10-17 Sept 1975 Madrid, Spain. 286 p.
5. HULL, R., R. J. SHEPHERD, and J. D. HARVEY. 1976.

Cauliflower mosaic virus: an improved purification procedure and some properties of the virus particles. J. Gen. Virol. 31:93-100.

6. KLEINSCHMIDT, A. K., A. BURTON, and R. L. SINSHEIMER. 1963. Electron microscopy of the replicative form of the DNA of the bacteriophage φX174. Science 142:961.

7. LAWSON, R. H., and E. L. CIVEROLO. 1976. Purification of carnation etched ring virus and comparative properties of CERV and cauliflower mosaic virus nucleic acids. Acta Hortic. 59:49-59.

8. LAWSON, R. H., and S. S. HEARON. 1974. Ultrastructure of carnation etched ring virus-infected Saponaria vaccaria and Dianthus caryophyllus. J. Ultrastruct. Res. 48:201-215.

9. PETTERSSON, U., C. MULDER, H. DELIUS, and P. A. SHARP. 1973. Cleavage of adenovirus Type-2 DNA into six unique fragments by endonuclease R·RI. Proc. Nat. Acad. Sci., USA 70:200-204.

10. PIRONE, T. P., G. S. POUND, and R. J. SHEPHERD. 1961. Properties and serology of purified cauliflower mosaic virus. Phytopathology 51:541-546.

11. SCHMIR, M., B. M. J. REVET, and J. VINOGRAD. 1974. Dependence of the sedimentation coefficient of the denatured closed circular DNA in alkali on the degree of strand unwinding. The absolute sense of supercoils. J. Mol. Biol. 83:35.

12. SHEPHERD, R. J. 1970. Cauliflower mosaic virus. No. 24 in Descriptions of Plant Viruses, Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England.

13. SHEPHERD, R. J. 1976. DNA viruses of higher plants. Pages 305-339 in M. A. Lauffer, F. B. Bang, K. Maramorosch, and K. M. Smith, eds. Advances in Virus Research, Vol. 20. Academic Press, New York. 360 p.

14. SHEPHERD, R. J., G. E. BRUENING, and R. J. WAKEMAN. 1970. Double-stranded DNA from cauliflower mosaic virus. Virology 41:339-347.

15. SOGO, J. M., R. PORTMANN, P. KAUFMANN, and T. KOLLER. 1975. Adsorption of DNA molecules to different support films. J. Microsc. 104:187-198.