Base Composition and Molecular Weight of DNA from a Frog Polyhedral Cytoplasmic Deoxyribovirus[1] (35026)

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(Introduced by A. Granoff)

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Amphibian polyhedral cytoplasmic deoxyribovirus (PCDV) is unique among animal viruses, not fitting into any of the currently recognized virus groups. PCDV, like poxviruses, is a DNA-containing virus which replicates in the cell cytoplasm. However, PCDV is smaller and physiochemically different from poxviruses (1–4), resembling more closely the insect iridescent viruses (Tipula, Sericesthis, and Chilo) (5). Although smaller, PCDV is also similar morphologically to African swine fever disease virus (6) and to lymphocystis disease virus of fish (7, 8).

Maes and Granoff (4) determined by cesium chloride buoyant density centrifugation that DNA, presumably viral, from the cytoplasmic fraction of PCDV infected cells had a base composition of 53% guanine plus cytosine (G + C). Smith and McAuslan (9) reported that DNA extracted from purified virus contained 56 to 58% G + C. Furthermore, they determined by rate sedimentation that the molecular weight of PCDV DNA was 130×10^6 daltons. In this study, we have estimated the mean amount of DNA per PCDV virion using electron microscopic and chemical techniques. We have also determined the base composition and molecular weight of DNA from purified virus.

Materials and Methods. Viruses and Isotopic Labeling of Viral DNA. The frog virus 3 isolate of PCDV (1, 2), grown in fathead minnow (FHM) cells (10) by procedures previously described (2), was used in all experiments.

PCDV DNA was ^{3}H-labeled by the following procedure. Confluent monolayers of FHM cells grown in 75-cm^2 plastic flasks (Falcon Plastics, Los Angeles, Calif.) were infected at 10 PFU/cell. Two hr after infection, 20 ml of Eagle's minimum essential medium with 2% heat-inactivated fetal calf serum and 5 μCi/ml of thymidine-methyl-^{3}H (Schwarz BioResearch; sp act 11.9 Ci/mmole) was added/culture. The virus was harvested after 2-days incubation at 23 to 25°.

A gift of ^{14}C-labeled T4 coliphage was generously made by Dr. Herbert L. Ennis of the Roche Institute for Molecular Biology. The labeling of T4 DNA was accomplished by propagating T4 in *E. coli* B207 in medium containing 0.25 μCi/ml of uracil-2-^{14}C (sp act, 7.21 mCi/mmole). Purification of T4 was by the method of Thomas and Abelson (11).

Base Composition of PCDV DNA. The method of Maes and Granoff (4) was used to extract DNA from purified virus. DNA was dissolved at 15–20 μg/ml in standard saline citrate solution (0.15 *M* NaCl, 0.015 *M* sodium citrate) and its melting profile obtained by the method of Marmur and Doty (12). The DNA solution was heated by circulating ethylene glycol in water from an electrically heated bath through the standard jacketed cell housing of a Zeiss PMQII spectrophotometer. A thermistor immersed in the DNA solution measured its temperature. The optical density values recorded at the various temperatures were corrected for thermal expansion of DNA solvent. Air bubbles were evacuated from DNA samples by bubbling with helium gas.

Molecular Weight of PCDV DNA. a. Zone

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centrifugation. Purified PCDV (^{3}H-labeled DNA) and T4 (^{14}C-labeled DNA) were diluted into a solution consisting of 2 mg/ml of Pronase (grade B, Calbiochem.), 9% (w/w) sucrose, 1% deoxycholate, 0.2 *M* NaCl, 0.001 *M* EDTA, and 0.001 *M* potassium phosphate, pH 7.1 (13). This reaction mixture (0.2 ml) was then carefully layered on top of a preformed, linear, 4.8 ml, 15 to 30% (w/w) sucrose gradient containing 1% sodium dodecyl sulfate, 0.1 *M* NaCl, 0.001 *M* EDTA, and 0.001 *M* potassium phosphate, pH 7.1. The gradients were covered with parafilm and incubated for approximately 12 hr at 23 to 25° to release DNA from virus. Gradients were then centrifuged in a Spinco model L-2 centrifuge (SW 39 rotor) at 25,000 rpm and 4 hr at 25°. Gradients were collected from the tube bottom in 3 drop fractions (calculated to contain 51 μl) on Whatman No. 1, 2.5-cm filter paper disks. Filter paper disks containing gradient fractions were processed for scintillation counting by the method of Bollum (14). Samples were counted in a model 3310 Packard Tri-Carb spectrometer, set for double label counting, using Liquifluor (New England Nuclear) in toluene as the scintillation fluid. Results were corrected for the 15% spillage of counts from the ^{14}C- to the ^{3}H-channel. Molecular weight of PCDV DNA was determined from sedimentation data by the method of Burgi and Hershey (15).

b. Electron microscopy. DNA was prepared and added to Formvar-coated grids as described by Hyde *et al.* (16). DNA was shadowed at right angles with uranium oxide and photomicrographs were taken with a Siemens Elmiskop 1 using double-condensor illumination at 40 kV accelerating voltage. Negatives were projected onto tracing paper and contour lengths of DNA molecules were determined with a map measurer.

Average Weight of DNA per PCDV Virion. Numbers of particles in purified virus preparations were determined by electron microscopy (17, 18). Quantities of DNA per known number of PCDV virions were obtained by use of the diphenylamine reaction (19). High molecular weight calf thymus DNA was used as a standard.

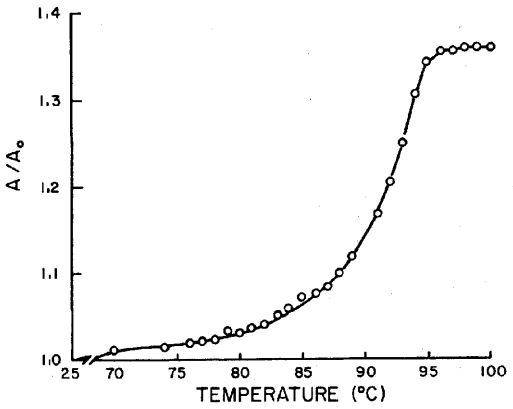

FIG. 1. Melting profile of PCDV DNA. PCDV DNA was dissolved at 15 μg/ml in standard saline citrate (pH 7.0) and the temperature of the solution was raised approximately 1° every 10 min. A/A_0 is the ratio obtained when the absorbance (260 mμ) of a DNA solution at any given temperature (A) is divided by its absorbance at 25° (A_0).

Results. A melting profile of PCDV DNA is shown in Fig. 1. In this experiment, the T_m (midpoint of the increase in absorbance at 260 mμ accompanying the melting of double stranded DNA) of PCDV was 91.3°. Additional melting experiments yielded T_m values for PCDV DNA varying between 91.3 and 91.8°. Employing the formula of Marmur and Doty (12):

$$G+C = \frac{T_m - 69.3}{0.41}$$

where G + C is the mole percent guanine plus cytosine, and T_m is defined above, PCDV DNA was calculated to contain 54 to 55% G + C, in reasonable accord with previously reported values (3, 9).

The molecular weight of PCDV DNA was estimated from its sedimentation behavior in neutral sucrose gradients. T4 coliphage DNA, reported to have a sedimentation coefficient of 62S and a molecular weight of 130×10^6 daltons (20, 21), was used as reference DNA. When centrifuged together in a neutral sucrose gradient, the distances sedimented by PCDV and T4 DNA's were identical (Fig. 2), indicating that their molecular weights were approximately the same. Therefore, PCDV DNA has a sedimentation

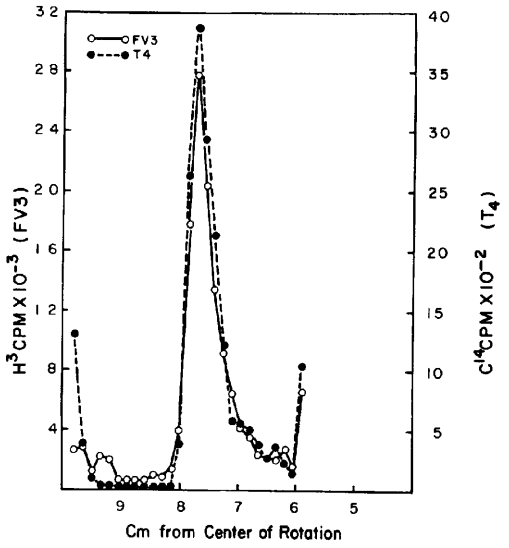

FIG. 2. Rate zonal centrifugation in a 15–30% (w/w) sucrose gradient of ^{3}H-labeled PCDV DNA and ^{14}C-labeled T4 DNA. Purified PCDV and T4 virions were carefully added to the top of the gradient and their DNA was released prior to centrifugation as discussed in the text.

coefficient of 62S and a molecular weight of about 130 $\times$ 10^6 daltons.

Gradients received from 0.05 to 0.2 μg of PCDV DNA and 0.75 μg of T4 DNA. Quantities of DNA added to gradients were calculated from electron microscopy particle counts on the basis that individual PCDV and T4 particles contained an average of 2.16 $\times$ 10^{-16} g of DNA (130 $\times$ 10^6 daltons). At these DNA concentrations, the rate of sedimentation of T4 or PCDV DNA in neutral sucrose were unaffected, regardless of whether DNA species were centrifuged alone or in combination.

To insure that DNA was released from PCDV and T4 virions under the conditions of incubation specified in Methods, gradient fractions containing peaks of radioactivity were tested for their susceptibility to deoxyribonuclease (DNase). Incubation with 25 μg/ml of pancreatic DNase I for 1 hr at 37°, rendered radioactivity in peak fractions about 90% acid soluble.

Attempts to more rigorously characterize PCDV DNA by analytical ultracentrifugation were unsuccessful presumably because of the fragility of the large genome to shearing forces. Even though precautions were taken to minimize shear during extraction, DNA extracted from virions and purified by methylated albumin–kieselguhr column chromatography had a lower sedimentation coefficient (approximately 40S) than did DNA released from virions on top of sucrose gradients (approximately 62S). The size variability of extracted DNA molecules was confirmed by electron microscopic measurement. Although contour lengths of DNA molecules were variable, the largest DNA molecules were computed to have molecular weights of about 130 $\times$ 10^6 daltons, in agreement with values obtained by zonal centrifugation. No evidence for circularity of PCDV DNA molecules was obtained by electron microscopy. An electron micrograph of PCDV DNA is shown in Fig. 3.

An estimation of the mean amount of DNA per PCDV virion was made using the procedure of Bellett and Inman (22). Two preparations of purified virus were found by electron microscopy to contain 2.1 $\times$ 10^{11} and 8.0 $\times$ 10^{11} particles/ml. By use of the diphenylamine reaction, these virus preparations were calculated to contain, respectively, 39 and 205 μg of DNA/ml of virus, equivalent to an average of 1.86 $\times$ 10^{-16} g (112 $\times$ 10^6 daltons) and 2.56 $\times$ 10^{-16} g (154 $\times$ 10^6 daltons) of DNA/virion. These values bracket the molecular weight of 130 $\times$ 10^6 daltons obtained for PCDV DNA by electron microscopy and by present and previous sedimentation studies (9). Thus, it is likely that each virion contains a single linear molecule of DNA. Unequivocal proof of this conclusion has not been obtained because of the susceptibility of PCDV DNA to shear.

Discussion. Based on these and previous results, the genome of PCDV is among the largest of all DNA viruses of animals, falling in size between that of the poxvirus and herpesvirus groups. As mentioned previously, PCDV is similar in size and morphology to the insect iridescent viruses (Tipula, Sericesthis, and Chilo). DNA from PCDV and the iridescent viruses is also of similar molecular weight (130 $\times$ 10^6 daltons) (22). However,

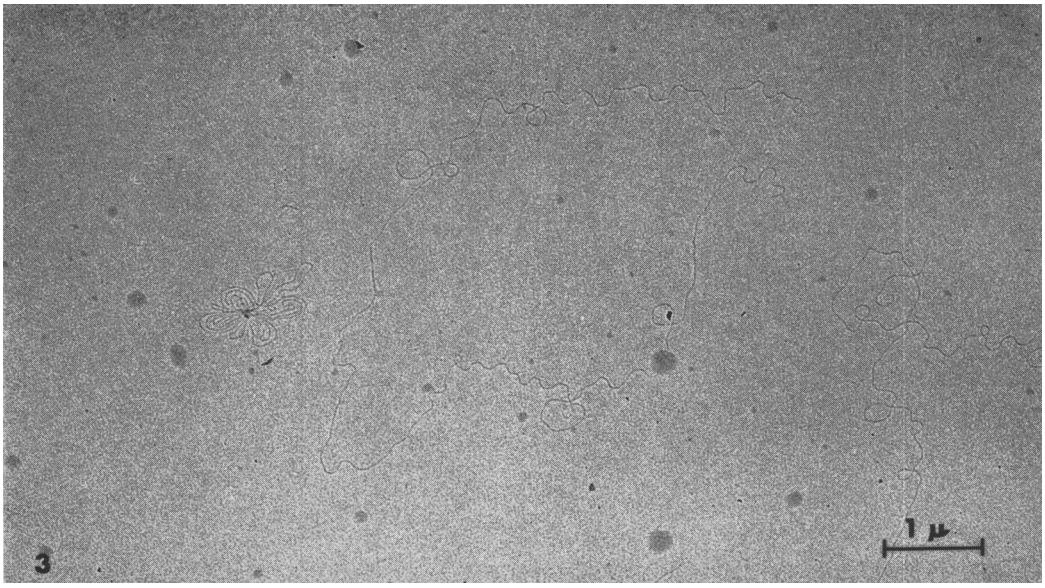

FIG. 3. PCDV DNA shadowed with uranium oxide; ×17,000.

these viruses differ in that PCDV acquires an envelope at cytoplasmic membranes and is ether sensitive, while the iridescent viruses are unenveloped and ether stable (2, 3, 5). Furthermore, DNA of the iridescent viruses has a lower G + C content (28–32%) than does PCDV (54–55%) (22). Also, RNA from the iridescent viruses does not hybridize with PCDV DNA (23). Considering these additional parameters, PCDV and the insect iridescent viruses do not appear to be closely related.

Summary. Frog PCDV DNA was estimated from its melting profile to contain 54–55% G + C. The molecular weight of PCDV DNA was confirmed to be about 130 $\times$ 10^6 daltons from its sedimentation behavior in neutral sucrose and by contour length measurements. The mean content of DNA per PCDV virion in two preparations of purified virus was 1.86 $\times$ 10^{-16} g (112 $\times$ 10^6 daltons) and 2.56 $\times$ 10^{-16} g (154 $\times$ 10^6 daltons). Data suggest that PCDV virions contain a single linear double-stranded molecule of DNA.

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