The Characterization of Varicella-zoster Virus DNA

(Accepted 1 October 1971)

Varicella-zoster (V-Z) virus was first isolated by Weller (1953) and has been classified as a group B cell-associated herpesvirus of man (Melnick et al. 1964). The cell-associated nature of the virus has made it difficult to obtain infectious virus from tissue culture and to prepare large quantities of virus particles for the characterization of the virion and its DNA. In this report we describe the successful purification of V-Z virus and define some of the properties of its DNA.

Two strains of V-Z virus were used in this study. Strain TC 182 was isolated from a child who had expired with generalized varicella (Benyesh-Melnick, Rosenberg & Watson, 1964), and strain EY was obtained from a patient with generalized herpes zoster infection (Rapp & Benyesh-Melnick, 1963). Both strains were propagated in human embryonic lung (HEL) fibroblasts as described previously (Rapp & Benyesh-Melnick, 1963). Intact infected cells were mixed with freshly trypsinized normal fibroblasts at a ratio of 1:10. The resulting cell suspensions were seeded in 16-oz prescription bottles with 30 ml. Eagle's medium containing 10% foetal bovine serum (FBS), 0.075% NaHCO₃, and gentamycin sulfate (50 μg./ml.), and allowed to form monolayers. Under these conditions complete monolayers contained approximately 5 × 10⁶ cells and revealed 80 to 90% c.p.e., within 3 to 4 days after seeding. Stocks of infected cells were kept at −195°C in Eagle's medium with 10% FBS and 10% dimethylsulfoxide.

To obtain large amounts of virus for characterization of the DNA, 50 bottles of HEL fibroblasts infected with strain EY were used. When the monolayers exhibited 80 to 90% c.p.e. EDTA was added to a final concentration of 0.005 M, intracellular virus was released into the medium by freezing and thawing the cultures, and cell debris was pelleted by centrifugation at 2500g for 40 min. The pellet was resuspended in a small amount of medium, frozen and thawed, sonicated for 45 sec. (in a Raytheon sonic oscillator) and centrifuged as above. Virus was precipitated from the pooled supernatant fluids by ammonium sulphate (39%, w/v) and purified through discontinuous sucrose gradients (30%-60%) followed by a final centrifugation in CsCl density gradients (ρ = 1.19 to 1.50 g./cm.³). In the CsCl density gradients two virus bands were readily distinguished. The upper, more diffuse band (ρ = 1.274 g./cm.³) contained approximately 85% enveloped virus particles, as determined by electron microscopy (McCombs, Benyesh-Melnick & Brunschwig, 1966), while the lower, sharp band (ρ = 1.294 g./cm.³) consisted of about 95% naked particles. The virus particles seen under the electron microscope exhibited morphology typical of V-Z virus (Almeida, Howatson & Williams, 1962). For the isolation of DNA, both virus-containing bands (approximately 10¹¹ to 10¹² particles) from the CsCl gradients were pooled and dialyzed against tris EDTA buffer (0.01 M tris-chloride, pH 7.4; 0.1 M NaCl and 0.001 M EDTA). To 10 ml. of virus suspension 0.2 ml. diethyl pyrocarbonate (Solymosy et al. 1968), 0.1 ml. 2-mercaptoethanol and 0.1 ml. 0.5 M EDTA were added as nuclease inhibitors. The particles were disrupted by sodium lauryl sarcosinate (0.3%) and deproteinized with a phenol m-cresol mixture (Biswa & Benyesh-Melnick, 1969). After deproteinization, the aqueous phase, containing the virus DNA, was dialyzed against 0.015 M NaCl + 0.0015 M Na citrate (0.1 × SSC) (Ludwig et al. 1971). The E₂₆₀/E₂₈₀ ratios of greater than 1.9:1 indicated a minimal protein contamination in these DNA preparations.
The buoyant density of the virus DNA was determined by analytical ultracentrifugation in a Spinco Model E centrifuge equipped with a photoelectric scanner. Approximately 2 μg of DNA was centrifuged to equilibrium in neutral CsCl at 44,000 rev./min. for 20 hr at 25°, and resolved as a sharp symmetrical peak (Fig. 1 A). The buoyant density of the DNA was calculated to be 1.705 g./cm.³ from repeated runs of different preparations using pseudorabies virus DNA (ρ = 1.731 g./cm.³) (Fig. 1 B) and E. coli DNA (ρ = 1.710 g./cm.³) (Fig. 1 C) as density markers. Assuming that there are no abnormal bases present, this density corresponds to a G + C content of 46% (Schildkraut, Marmur & Doty, 1962). When 3 μg. of the virus DNA was heated at 100° for 10 min. in 0.1 × SSC, it was denatured and subsequently banded as a single peak at a density of 1.720 g./cm.³ in CsCl (Fig. 1 D). This indicated that the V-Z virus DNA was double-stranded, but the two strands could not be resolved by the method employed.

Since the EY strain of V-Z virus used in the above analysis of the DNA was originally isolated from a case of herpes zoster, it was of interest to determine if strain TC 182, isolated from a patient with varicella, contained DNA of the same density. For this purpose, intracellular DNA from cells infected either with strain EY or with strain TC 182 was centrifuged to equilibrium in CsCl in an analytic ultracentrifuge (Ludwig et al. 1971). With both strains, virus DNA peaks were resolved at a density of 1.705 g./cm.³ (Fig. 2), identical to that of
DNA from purified virus. Figs. 2 A and 2 B show representative tracings in which the virus DNAs are resolved clearly from host-cell DNA and marker DNAs of pseudorabies virus and E. coli. As a control, DNA from uninfected HEL fibroblasts banded only as a single peak with a buoyant density of 1.697 g./cm.³ (Fig. 2 C).

In this study we have determined the buoyant density of V-Z virus DNA to be 1.705 g./cm.³. The finding that virus DNA from cells infected with strains EY and TC 182 have the same density provides further evidence that the immunologically related viruses isolated from the clinical entities of varicella and herpes zoster (Weller, Witton & Bell, 1958) are the same. These results are clearly different from those of Gershon, Casio & Brunell (1971) who reported a buoyant density of 1.717 g./cm.³ for V-Z virus DNA. Our observation that V-Z virus DNA has a density (ρ = 1.705 g./cm.³) close to that of cell DNA (ρ = 1.697 g./cm.³) may explain past difficulties in resolving virus DNA from cell DNA (Plummer et al. 1969).

Our findings demonstrate that V-Z virus DNA has a different buoyant density from those reported for other herpesviruses of man, such as herpes simplex and cytomegalovirus (Plummer et al. 1969; observations in our laboratory). In addition, the estimated G + C content of 46% for V-Z virus DNA is significantly lower than that of any other previously reported herpesvirus of man (Plummer et al. 1969).
Short communications

We are grateful to Dr R. M. McCombs for the electron microscopic analysis of the preparations. We thank Tim Woodward, Ellen Morrison, Robert Striker and Catherine Green for excellent technical assistance. This work was supported by research Grant CA 04600 from the National Cancer Institute, Contract PH 43-68-678 with the Special Virus Cancer Program, training grant 5 T l A 1 7 4 from the National Institute of Allergy and Infectious Diseases, NIH, and by a supporting grant from Deutsche Forschungs-Gemeinschaft.

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(Received 21 June 1971)

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