A Study of Rabbit Kidney Vacuolating Virus and its DNA

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SUMMARY

Rabbit kidney vacuolating virus is similar to polyoma virus in the size and structure of the virus particle. The DNA of the virus is also similar to the DNA of polyoma virus, being supercoiled and circular with a molecular weight of approximately 3 million. The base composition of the DNA, 43 % guanine plus cytosine (GC), is intermediate between that of polyoma virus DNA, 48 % GC, and that of simian virus 40 DNA, 41 % GC. It is concluded that rabbit kidney vacuolating virus should be placed with polyoma virus and simian virus 40 as a member of the polyoma group of viruses.

INTRODUCTION

Rabbit kidney vacuolating (RKV) virus was originally isolated by Hartley & Rowe (1964) as a contaminant in some batches of Shope rabbit papillomata. The virus was shown to be similar in its general properties to polyoma virus and SV 40 and electron micrographs of the virus particles indicated a close similarity in size and general appearance between the two viruses (Hartley & Rowe, 1964; Chambers, Hsia & Ito, 1966). The inhibitory effect of bromodeoxyuridine and iododeoxyuridine on the growth of the virus was consistent with RKV virus being a DNA containing virus (Hartley & Rowe, 1964). Ito, Hsia & Evans (1966) were also able to extract infectious DNA from RKV virus.

This study was undertaken to provide a more detailed comparison of RKV virus with polyoma virus. The DNA of the virus was examined by analytical centrifugation and electron microscopy.

METHODS

Virus production and purification. The virus stock (strain 443, pool 903) was kindly supplied by Drs Hartley and Rowe and was propagated in primary and secondary cultures of domestic rabbit kidney. The kidneys were taken from 15- to 18-day-old rabbits. Cultures were grown in Eagle's medium containing twice the standard concentration of amino acids and vitamins plus 10 % tryptose phosphate broth (Difco) and 5 % (v/v) horse serum (heated to 56° for 30 min.) at 37° in a humidified incubator flushed with 5 % CO₂. When the cultures were nearly confluent they were infected and harvested 3–5 days later when the monolayers began to come off the glass. The cells remaining attached to the glass were scraped off into the medium, the suspension centrifuged (3000g for 15 min.) and the pellet resuspended in phosphate buffered saline and frozen and thawed three times. The virus was extracted from the cell debris by treatment with receptor destroying enzyme and sodium deoxycholate
(0.25% w/v) for 3 hr at 37°. It was necessary to use both receptor destroying enzyme and deoxycholate to obtain maximum yields of virus from the infected cell debris.

The virus was concentrated by centrifugation (66,000g for 2 hr) and purified by density gradient centrifugation. One ml. of virus suspension was layered on 3 ml. of CsCl solution (40%, w/w) containing 2-amino-2-hydroxymethyl propane 1:3: diol (tris) buffer (0.05M, pH 8.0). After centrifugation (73,500g for 20 hr) the tubes were examined in a beam of light and opalescent bands collected. The dense band (density = 1.32 g./ml.) was found to contain 'full' virus particles and was used for the experiments described below.

Polyoma virus, polyoma virus DNA and human papilloma virus were prepared as previously described (Crawford, 1962, 1963; Crawford & Crawford, 1963).

**DNA extraction.** DNA was extracted from purified virus suspensions by heating with sodium dodecyl sulphate (2%, w/v) at 60° for 10 min. The detergent was precipitated by making the solution 1M with NaCl. After 18 hr at 4° the precipitate was spun off and the DNA sedimented through CsCl (45%, w/w). The DNA was then resuspended in tris buffer (0.01M, pH 8-0) containing versene (mM) and stored at 4°.

**Analytical centrifugation.** The band centrifugation method was used to examine the sedimentation behaviour of RKV virus DNA in neutral and alkaline CsCl (Vinograd et al. 1963a, b). The conditions used were as follows: 12 mm. cell with 4° Kel-F centrepiece, 0.6 ml. bulk solution and 5 to 20 µl. lamellar volume. The bulk solution was CsCl (density = 1.5 g./ml.) containing Tris buffer (0.05M, pH 8.0) or phosphate buffer (0.04M, pH 12.5). Samples were centrifuged at 42,040 rev./min. and 20° in a Spinco model E analytical ultracentrifuge. Ultraviolet absorption photographs were taken and scanned with a Joyce Loebl microdensitometer.

Equilibrium density gradient centrifugation (Meselson, Stahl & Vinograd, 1957) in neutral and alkaline CsCl was used to study the properties of native and denatured virus DNA. The CsCl solutions were as described above except that the density was increased to approximately 1.71 g./ml. Samples were centrifuged at 44,770 rev./min. and a small amount of *Clostridium welchii* or *Micrococcus lysodeikticus* DNA was included to act as a marker of known density.

**Electron microscopy.** Virus suspensions were examined by the negative-staining method using 2% (w/v) phosphotungstic acid adjusted to pH 6.8 with KOH. Micrographs were taken at a magnification of 40,000× with a Siemens Elmiskop 1.

Virus DNA was examined by the protein monolayer technique of Kleinschmidt & Zahn (1959). The DNA-containing protein film was prepared by spreading 0.3 ml. of ammonium acetate (1.0M) containing 3 µg./ml. virus DNA and 0.1 mg./ml. cytochrome c (Sigma type VI) on a hypophase of ammonium acetate (0.1M, pH 8.0). Areas of the film were picked up on seven-hole platinum specimen mounts and shadowed with platinum at an angle of 7° while rotating. Micrographs were taken at an indicated magnification of 10,000×. A more accurate value for the actual magnification was obtained from a diffraction grating replica examined under identical conditions.

Contour lengths of circular DNA molecules were measured by projecting the image from the photographic plate on to paper at a magnification of 17× with a photographic enlarger, tracing the outline and measuring the tracing with a map measurer.
RESULTS

Morphology of the RKV virus particle

The morphology of particles of RKV virus is shown in Pls. 1 and 2. The particles are very similar in size to those of polyoma virus and distinctly smaller than those of human papilloma virus. The mixed preparation (Pl. 1, figs. 1, 2) shows clearly the difference in size between RKV virus and papilloma virus particles. The ratio of particle diameters, RKV virus: papilloma virus, is approximately the same as previously found for polyoma virus: papilloma virus, 1 : 1.2 (Crawford & Crawford, 1963). In both cases purified preparations of ‘full’ particles were used for the comparison. In addition to the usual ‘full’ and ‘empty’ particles, elongate forms of RKV virus were also found in unpurified virus preparations (Pl. 1, fig. 3). Similar elongate forms have been observed in some polyoma virus preparations (Howatson & Almeida, 1960).

When examined at high magnification single RKV virus particles presented a confused pattern. Hollow capsomeres could be distinguished with five or six nearest neighbours, but no one particle showed a sufficient number of clearly defined capsomeres to allow an unequivocal determination of the number of capsomeres between adjacent fivefold axes of symmetry and thus of the total number of capsomeres on the particle. A similar confusion of pattern has been shown by Klug & Finch (1965) to be common to the majority of particles of the polyoma-papilloma virus group and to arise from superposition of detail from both sides of virus particles having 72 capsomeres arranged on an icosahedral surface lattice with triangulation number $T = 7$. In such preparations a few particles are distinctive, displaying ‘eyes’ or ‘threefold terraced’ images (Klug & Finch, 1965). In Pl. 2, figs. 4a, 4c, 5a, 5c examples are shown of RKV virus particles displaying these distinctive patterns. Between the examples are shadowgraphs of Geodestix models of $T = 7$ (taken from Klug & Finch, 1965) in corresponding orientations. Our study was not sufficiently extensive to allow a definite decision on the number of capsomeres of the RKV virus particle but it is most likely that RKV virus is similar in structure to polyoma virus.

The physical properties of RKV virus DNA

The sedimentation behaviour of RKV virus DNA was first examined by band centrifugation (Vinograd et al. 1963a) in CsCl (density = 1.50 g./ml.) at pH 8.0. Two components were present in approximately equal amounts, with sedimentation coefficients of 8.2 S and 5.8 S. Under the same conditions polyoma virus DNA showed two components, 8.4 S and 5.8 S representing supercoiled circular and simple circular molecules respectively.

Under alkaline conditions normal DNA undergoes strand separation but in a circular molecule, such as polyoma virus DNA, the strands of the molecule remain entangled with each other and the molecule collapses into an extremely compact form which sediments very rapidly (Weil & Vinograd, 1963; Vinograd et al. 1965). In CsCl (density = 1.50 g./ml.) at pH 12.5 RKV virus DNA sedimented as two components, 24.4 S and 7.2 S, as compared with polyoma virus DNA 25.0 S and 7.4 S under the same conditions. The similarity of the values would indicate that RKV virus DNA is circular, like polyoma virus DNA. The fast component is therefore taken to be collapsed intact molecules and the slow component to be a mixture of
circular and linear single strands which have not been resolved from each other here.

The collapsed molecules of circular DNAs have an anomalously high buoyant density in alkaline CsCl because of their compact centrifugation. For example polyoma virus DNA gives two bands at densities of 1.784 and 1.766 (Weil & Vinograd, 1963). In CsCl at pH 12.5 RKV virus DNA gave two bands at densities of 1.778 and 1.760. The lighter band consists of circular and linear strands from molecules in which a single strand scission had occurred allowing the two strands to separate. The denser band comprises collapsed intact molecules as explained above. This is further evidence for the circularity of RKV virus DNA.

Equilibrium density gradient centrifugation at neutral pH was used to determine the base composition of RKV virus DNA. The content of guanine plus cytosine (GC) in double stranded DNA is linearly related to the buoyant density of the DNA (Schildkraut, Marmur & Doty, 1962). The base composition of RKV virus DNA, calculated from its density relative to Micrococcus lysodeikticus DNA, was 43% GC.

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\begin{align*}
\text{Length (\mu)} & \quad \text{No. of measurements} \\
1.0 & \quad 15 \\
1.2 & \quad 10 \\
1.4 & \quad 5 \\
1.6 & \quad 20 \\
1.8 & \quad 15
\end{align*}
\]

Fig. 1. Histogram of the length distribution of RKV virus DNA molecules. A total of 68 circular molecules was measured. The length in microns is given by the abscissa and the number of molecules for each interval of 0.1 \(\mu\) is given by the ordinate.

**Electron microscopy of RKV virus DNA**

To obtain further information on the size and shape of RKV virus DNA samples were prepared by the protein monolayer method of Kleinschmidt & Zahn (1959). The samples showed both circular and supercoiled molecules (Pl. 2, figs. 6, 7). The mean contour length of 68 circular molecules was 1.44 ± 0.14 \(\mu\) with the distribution shown in Fig. 1. Under the same conditions the mean length of 219 molecules of polyoma virus DNA was found to be 1.52 ± 0.14 \(\mu\). The two DNAs are therefore not significantly different in size. Assuming a mass per unit length of 1.97 daltons per micron (Langridge et al. 1960) these lengths correspond to a molecular weight of approximately
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3 million daltons. The electron micrographs showed clearly that some of the molecules of RKV virus DNA were supercoiled, resembling polyoma virus DNA in this respect also.

DISCUSSION

Rabbit kidney vacuolating virus appears to resemble polyoma virus very closely with respect to the size and morphology of the virus particle and the size and structure of the DNA. The virus particle is distinctly smaller than that of human papilloma virus. This is consistent with the conclusion of Hartley & Rowe (1964) that RKV virus was a contaminant in the rabbit papillomata, rather than a variant papilloma virus. The size of the DNA of RKV virus, approximately 3 million molecular weight, is also quite different from that of papilloma viruses, 5-3 million (Crawford, 1965). In its physical properties RKV virus DNA also resembles polyoma virus DNA closely. The base composition found for RKV virus DNA, 43 % GC, may be compared with that of SV 40 DNA, 41 % GC (Crawford & Black, 1964) and polyoma virus DNA, 48 % GC (Crawford, 1962).

Direct observation of RKV virus DNA by electron microscopy confirmed its similarity to polyoma virus DNA. Some of the molecules could be seen to be twisted i.e. supercoiled, as had previously been observed for polyoma virus DNA by Vinograd et al. (1965) and for SV 40 DNA by Crawford, Follett & Crawford (1966). Although RKV virus has not been reported to cause turnouts its properties are such that it should be placed with the tumour viruses polyoma and SV 40 as a member of the polyoma group of viruses.

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REFERENCES


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EXPLANATION OF PLATES

The bars in Pl. 1, figs. 1 to 3 indicate 1000 Å.

Fig. 1. Purified preparation of RKV virus negatively stained with potassium phosphotungstate.

Fig. 2. Mixed preparation of RKV virus and human papilloma virus. The human papilloma virus particles are indicated by arrows.

Fig. 3. Elongate form of RKV virus.

Fig. 4a, c. Particles of RKV virus displaying 'eyes', taken from a mixed preparation. The arrowed particles of RKV are to be compared with the shadowgraph of a Geodestix model of T = 7 shown in Fig. 4b. The other, larger, particles are human papilloma virus.

Fig. 5a, 5c. Particles of RKV virus displaying 'threefold terraced' pattern. Each particle shows three prominent capsomeres in the centre of the particle, surrounded by two distinct 'terraces' (Klug & Finch, 1965). The arrowed particles are to be compared with the shadowgraph of a Geodestix model of T = 7 viewed down an axis of threefold symmetry (Fig. 5b).

Fig. 6. Circular molecule of RKV virus DNA prepared by the method of Kleinschmidt & Zahn (1959).

Fig. 7. Supercoiled circular molecule of RKV virus DNA prepared as for Fig. 6.