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PRELIMINARY REPORT

SEPARATION OF ADENOVIRUS DNA'S OF DIFFERENT MOLECULAR LENGTH BY AGAROSE-GEL ELECTROPHORESIS

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Agarose-gel was used as supporting medium in electrophoresis of linear DNA's of adenovirus type 5. It was found that the migration rates of these linear DNA's were inversely related to their relative molecular length assessed on a sucrose density gradient. That is, the rate of migration decreased with increase in size of the DNA.

P^{32}-labelled DNA was extracted from purified adenovirus type 5 by the method of GREEN and PIÑA (1964). H^3-labelled DNA was extracted by the procedure of SHEININ (1966) from hamster kidney cells labelled for 2 hours with H^3-thymidine 27 hours after infection with adenovirus type 5. In both methods, the extraction of DNA consisted essentially of treatment with proteolytic enzyme (papain or pronase), sodium lauryl sulphate and phenol. All the DNA synthesized at this period seemed to be viral DNA by the CsCl equilibrium centrifugation method as demonstrated in Fig. 1.

Electrophoresis was carried out as follows. Agarose-gel of 1.0% strength containing 0.05 M barbital buffer, pH 8.5, was melted and poured onto a 12.0×15.0 cm glass slide to give a layer of uniform thickness. Six holes of 5 mm in diameter were cut out 1.5 ~ 2 cm apart. To avoid possible contamination of adjoining samples of DNA during migration, the samples were completely saparated from each other by cutting out and removing a strip of agarose-gel

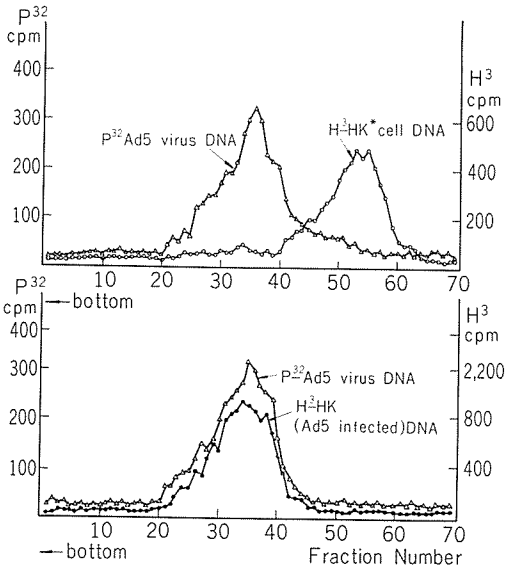

FIGURE 1 CsCl Equilibrium Centrifugation.
CsCl was added to labelled DNA solution (saline-citrate) to give a density of approximately 1.7 g/cm³ and the solution was buffered at pH 8 with 0.015 M Tris; 4.5 ml of this solution was transferred to Lusteroid tubes and was centrifuged at 35,000 rpm for 40 hours at 4°C in the SW-39 rotor of the Spinco model L. At the end of run, fraction was collected drop by drop on papers. They were dried and washed with 5% cold trichloroacetic acid and ethanol, and the radioactivity was assayed by liquid scintillation counter.
* HK: Hamster Kidney Cell

(0.2×15 cm) between the samples. Samples for analysis (50 μl) containing about 1 μg DNA were put into the holes without admixture with gel. Papers moistened with buffer connected the gel sheet to the electrodes. The apparatus was put in an air-tight box to prevent dehydration of the gel. A field strength of 7 volts/cm length of gel was applied. After electrophoresis for 2 hours, the gel was cut into segments (0.5×2.0 cm) which were transfered to vials. Bray's scintillation fluid (Bray, 1960) was added and the radioactivity was assayed in a Packard spectrophotometer.

A neutral sucrose density gradient was set up with a linear concentration of 5% to 20% sucrose in 0.1 M NaCl, 0.05 M phosphate buffer (pH 6.7). A sample containing up to 0.2 μg of DNA in 0.1 or 0.2 ml of 0.1 M buffered saline was layered onto 4.8 ml of the gradient and spun at 35,000 rpm for 3 hours in a SW 39 rotor of a Spinco model L centrifuge at 10°C. The sample was fractionated by drop collection.

Fig. 2 (a) (b) show the results of electrophoresis of DNA of adenovirus type 5 and DNA from adenovirus-infected hamster kidney cells on agarose-gel. The slow moving fraction was intact viral DNA (L) and the fast moving fraction seemed to be fragmented viral DNA (S). When the same sample used for Fig. 2 (b) was applied to the sucrose density gradient, the result given in Fig. 2 (c) was obtained. The fraction of large intact molecules (L) sedimented fast and the fraction of small fragmented molecules (S) sedimented slowly.

The relative molecular length of DNA (L) has been estimated from the distance sedimented (D) in a neutral sucrose density gradient. (BURGI and HERSHEY, 1963, STUDIER, 1965)

$$\frac{D_1}{D_2} = \left(\frac{L_1}{L_2}\right)^{0.35}$$

The ratio of the distances migrated by fractions L (D'_L) and S (D'_S) in agarose-gel were compared with that in the sucrose density gradient.

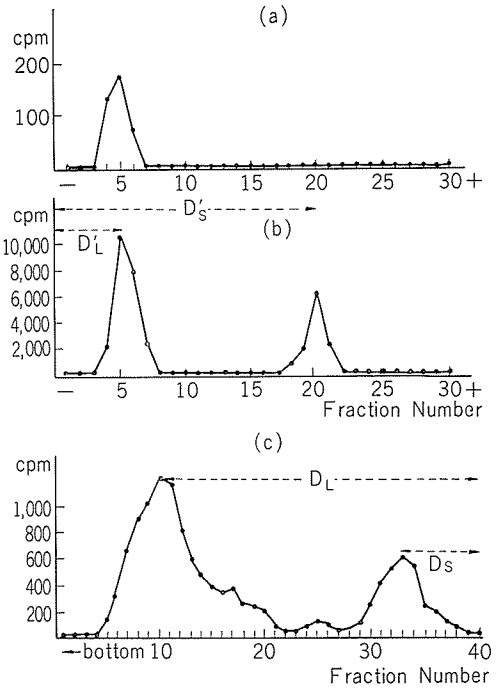

FIGURE 2 (a) Agarose-gel Electrophoresis of P^{32}-DNA of Adenovirus Type 5.
(b) Agarose-gel Electrophoresis of H^3-DNA of Hamster Kidney Cells infected with Adenovirus Type 5.
(c) Sucrose Density Gradient of H^3-DNA of Hamster Kidney Cells infected with Adenovirus Type 5. (The same sample used for (b)).

Then

$$\frac{D'_L}{D'_S} = \frac{5}{20} \qquad \frac{D_L}{D_S} = \frac{30}{7}$$

Therefore empirically,

$$\frac{D'_L}{D'_S} \fallingdotseq \frac{D_S}{D_L}$$

Thus the relative molecular length of DNA may be roughly estimated from the distance migrated in agarose-gel electrophoresis.

THORNE (1966) reported separation of polyoma DNA (circular) and mouse embryo cell DNA (linear) by agar-gel electrophoresis. He

attributed this separation to the difference in the molecular dimensions of the two DNA's. Agarose is the main component of agar, but agarose is almost free of various electrokinetic chemicals contained in agar, which might interact with DNA during electrophoresis and disturb the migration pattern. Thus agarose seemed suitable for separation of adenovirus DNA's of different molecular lengths.

The advantages of the agarose-gel electrophoresis method over the sucrose density gradient method are as follows.

1) At least 6 samples can be handled simultaneously.
2) Conditions for electrophoresis can easily be set uniformly, rendering variations in conditions between samples negligible.
3) A single labelling with isotope is enough for all samples, as the reference sample can be set separately and migrates simultaneously with the experimental samples.

Further study is necessary to see whether the agarose-gel electrophoresis method is applicable to other species of DNA.

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