Gel Filtration on Agarose

in the Separation of Adeno-associated Viruses from Adeno-viruses and Their Structural Components

(Accepted 23 June 1969)

Gel filtration on agarose (agar) has been used to separate viruses differing in size (Bengtsson & Philipson, 1964; Čech, 1962; Fridborg et al. 1965; Steere & Ackers, 1962). We therefore tried it for the separation of adeno-associated viruses (AAV) from adenoviruses, including the structural components of the latter. The results presented here indicate that chromatography on Sepharose 2B (Pharmacia, Uppsala, Sweden) represents a useful step for such separation.

The source of AAV and adenovirus was similar to that used in previous studies (Hoggan, Blacklow & Rowe, 1966) with adenovirus type 7, strain LLE46+, containing the adenovirus SV40 hybrid and AAV type 1. The viruses were grown in African green monkey kidney cells. The preparation of [3H]thymidine labelled adenovirus type 7 and the measurement of radioactivity are described in a subsequent paper (A. R. Neurath, B. A. Rubin & R. W. Hartzell, 1969, in preparation). The titration of complement fixing antigens, adenovirus haemagglutinins (HA) and incomplete haemagglutinins (IHA), rate zonal centrifugation in sucrose gradients and the calculation of approximate values of sedimentation coefficients (S20,w) were described by Neurath, Rubin & Stasny (1968). Human convalescent serum which did not contain CF antibodies against AAV, and guinea pig antiserum against AAV type 1 (supplied, respectively, by Drs R. M. Chanock and M. D. Hoggan of the National Institutes of Health, Bethesda, Maryland) were used for the titration of adenovirus and AAV complement fixing antigens.

Adenovirus, AAV and their complement fixing antigens were concentrated and partly purified from crude tissue culture fluids by precipitation with half-saturated ammonium sulphate. This procedure resulted in complete precipitation of the virus antigens. After centrifugation, the precipitate was redissolved in the minimal volume of 0.01 M-tris buffer, pH 7.0. Two to three ml. were applied on the bottom of K25/45 columns equipped with upward flow adaptors (Pharmacia, Uppsala) and filled with Sepharose 2B. 0.01 M tris buffer containing 0.14 M-NaCl was used as eluant. Fractions of 5 ml. were collected.

Specimens for electron microscopy were placed on carbon grids and negatively stained with 2 % (w/v) uranyl acetate. A RCA EMU-3H electron microscope with double condenser illumination was used.

Results presented in Fig. 1 show the distribution of AAV complement fixing antigens, adenovirus complement fixing antigens and IHA in fractions obtained after gel filtration. While IHA was recovered only in a few fractions, complement fixing antigens titrated with antiserum against adenovirus were detected in all fractions following the void volume of the column. This was not surprising, since all adenovirus capsid antigens in all possible states of aggregation (intact virus particles, groups of capsomeres, HA and single capsomeres) are detected by the titration. The majority
of adenovirus complement fixing antigens, however, were recovered in the same fractions as IHA separately from the main (left) peak of AAV complement fixing antigen. The bulk of tissue culture fluid proteins was also recovered in these fractions. HA was detected in fractions corresponding to the void volume of the column and in fractions corresponding to the peak of AAV complement fixing antigen. The first HA peak represented adenovirus particles, and the second, complete HA (dodecons).

In a separate experiment, isotopically labelled, purified adenovirus was chromatographed on the same column. This was done to obtain additional information on the distribution of adenovirus particles in the fractions using a method other than electron microscopy or HA titrations. The corresponding distribution of isotopic label (also shown on Fig. 1) indicated that adenovirus particles were eluted from the column before AAV. Since previous experiments (Neurath et al. 1968) showed that purified adenovirus type 7 undergoes spontaneous partial degradation during storage, the distribution of label in the fractions cannot be expected to correspond exactly to the distribution of intact adenovirus. This would apply, preferentially, to the trailing edge of the peak of label which may represent DNA-containing breakdown products of the virus.

Further information about the separation of AAV and adenovirus was obtained by electron microscopical screening of the fractions (Pl. 1a, b; see also text to Fig. 1).
a. Pooled fractions 11 to 14 from a gel filtration experiment similar to that mentioned in Fig. 1, in which the elution pattern was shifted about 1 fraction to the left. Most adenovirus particles are intact.

b. Pooled fractions 19 to 23. Full and empty AAV particles, some adenovirus dodecons, virus particles and groups of capsomeres.

c. AAV particles from b after additional isopycnic density gradient centrifugation. Fractions corresponding to the density range of 1.372 to 1.420 g./cm³. Bar length 1000 Å.

A. R. NEURATH AND OTHERS

(Facing p. 452)
Although it was possible to separate AAV from the bulk of adenovirus particles and structural components some of these were detected in the same fractions as AAV. Additional purification of AAV was achieved by isopycnic centrifugation in CsCl gradients (Plate 1c). AAV complement fixing antigen was recovered from such gradients at a density range from 1.30 to 1.43 g./cm³ with a distinct peak at density 1.37 g./cm³.

Fig. 2. Rate zonal centrifugation (40,000 rev./min. for 1 hr) of AAV complement fixing antigens in sucrose gradients (10 to 25% sucrose in 0.072 M-NaCl, 0.075 M-phosphate, pH 7.2). Bottom, crude tissue culture material; middle, recentrifugation of samples corresponding to the leading edge of the complement fixing antigen peak; top, recentrifugation of samples corresponding to the trailing edge of the complement fixing antigen peak. Fraction 1 = bottom fraction 15 = top.

The appearance of two peaks of AAV complement fixing antigen, as well as the more gradual rise of the first (left) peak of this antigen, as compared with the steep rise of peaks corresponding to either IHA or isotopic label, suggested that AAV complement fixing antigens were heterogeneous with respect to size. This was confirmed by rate zonal centrifugation experiments using either crude tissue culture material (Fig. 2, bottom) or pooled fractions 21 to 26 obtained from gel filtration. When sufficient pooled fractions corresponding to parts of the broad peak of AAV complement fixing antigen (Fig. 2, bottom) were recentrifuged under identical conditions of rate
zonal centrifugation, clear evidence for the heterogeneity of these AAV complement fixing antigens was obtained (Fig. 2, middle and top). While the peaks of complement fixing antigens corresponding to the bottom and top parts of Fig. 2 had a position in the gradient corresponding to $S_{20, w}$ value of about 75 to 80 $S$, the position of the peak in the middle part of Fig. 2 corresponded to a $S_{20, w}$ value of about 110 to 120 $S$. Some complement fixing antigen from the crude tissue culture material remained at the top of the gradient.

We believe that the complement fixing antigens with the higher sedimentation coefficient represent intact AAV, while the 75–80 $S$ antigens probably represent deficient AAV particles, which may be analogous to such particles detected among the antiserum-precipitable components of bacteriophage $\phi X 174$ (Greenle & Sinsheimer, 1968). The AAV complement fixing antigens corresponding to the second (right) peak on Fig. 1 and those recovered on the top of sucrose gradients (Fig. 2, bottom) probably represent AAV capsomeres.

This investigation was supported in part by contract PH 43-68-1316 from the Vaccine Development Branch, National Institute of Allergy and Infectious Diseases.

The technical assistance of S. K. Vernon in electron microscopy and of Eve Tober in measurement of radioactivity is gratefully acknowledged.

We thank Dr H. W. Ruelius for permission to use the scintillation counter, J. F. Ongaro for reading the manuscript, Noreen Mullin for typewriting and P. Freimuth for drawings.

Department of Research and Development
Wyeth Laboratories, Inc.
Philadelphia, Pennsylvania 19101, U.S.A.

REFERENCES


(Received 30 April 1969)