The Soluble Hemagglutinins of Adenoviruses Belonging to Rosen's Subgroup III

I. The Rapidly Sedimenting Hemagglutinin

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With 6 Figures

(Received July 12, 1968)

Introduction

The hemagglutinating capacity of adenoviruses was first demonstrated by Rosen (1960), who also grouped these viruses according to the character of this biological activity. Serotypes 1, 2, 4, 5 and 6 formed subgroup III, since they were the only adenovirus types exhibiting partial agglutination of rat erythrocytes. Studies on the relationship of this activity to different soluble components separated by anion exchange chromatography have demonstrated that the complete HA's were related to the pentons. In contrast fibers reacted as incomplete HA's demonstrable only in the presence of antisera against the other serotypes belonging to Rosen's subgroup III (Klemperer and Pereira, 1959; Pereira and de Figueiredo, 1962).

Morphological characterization of pentons, which also carry the toxin activity, i.e. causing cell detachment (Everett and Ginsberg, 1958; Pereira, 1958, and Rowe et al., 1958), revealed that they represented vertex capsomers carrying projections. Fibers represented the isolated projections and could be prepared from pentons by trypsin treatment (Valentine and Pereira, 1965). In the subsequent presentation the structural components will be termed hexons, pentons, and fibers with a slight modification of the terminology proposed by Ginsberg et al. (1966).

Ultrastructural characterization of soluble complete adenovirus HA's has revealed the occurrence of dodecahedral aggregates of 12 pentons

1 Supported by grants from the Swedish Medical Research Council (Project no. K 67-16x-548-03 and B 68-16x-744-03C).
in serotypes 3 and 11 (Norrby, 1966a; Norrby, 1968b) and 9, 13, 15 and 19 (Norrby et al., 1967; Gelderblom et al., 1967) belonging to Rosen's subgroups I and II, respectively. In contrast, only one member of Rosen's subgroup III, serotype 4 (Wadell, Norrby and Schönning, 1967) has been found to exhibit the same principal composition of its soluble complete HA. After zonal centrifugation of the soluble components of this serotype two different incomplete HA's were recovered. A rapidly sedimenting component, identified in the electron microscope as isolated pentons (Wadell, to be published), displayed a sedimentation rate somewhat lower than hexons. Incomplete HA's of a similar nature have also been identified in preparations of serotypes 3, 9 and 11 (Norrby, 1966b; Norrby, 1968a; Norrby et al., 1967). However in centrifugation experiments with preparations of serotype 5 the complete HA was found to sediment slightly faster than hexons (Norrby and Wadell, 1967). This difference in the ratios of hexon/penton sedimentation rates was considered too large to be accounted for by the difference in lengths of the projections of the pentons of for example serotypes 4 and 5, 175 and 250 Å, respectively (Valentine and Pereira, 1965; Wadell, Norrby and Schönning, 1967).

The aim of this study has been to further characterize this complete penton HA of type 5 and the related serotypes 1, 2 and 6.

Methods

Preparation of virus material. The prototype strains "Adenoid 71", "Adenoid 6", "Adenoid 75" and "Tonsil 99" of adenovirus serotypes 1, 2, 5 and 6, respectively, passaged on HeLa cells between 13 and 22 times prior to arrival in this laboratory were studied.

Stock materials were prepared by propagation of virus in a human embryonic bone marrow cell line denoted MAS-A cells (Kjellén, 1961) or a human embryonic lung cell line, Lu 106 cells. For maintenance of the cells Parker's medium 199 containing 2% calf serum or Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate and 3% calf serum were used, respectively. Harvests were taken at an advanced stage of degeneration. All media contained 100 I.U. penicillin, 50 µg streptomycin and 50 µg kanamycin per ml. The pools of medium and cells were concentrated 10 to 20 times by forced dialysis using polyethyleneglycol (Carbowax 6000, Union Carbide Chemicals Co., U.S.A.). These concentrates were frozen and thawed three times and cell debris was removed by low speed centrifugation. "Non-soluble" components i.e. intact virions and empty capsids had been removed from the material used in most experiments by centrifugation at Pi 5.3 (20,000 r.p.m. rotor 40, Spinco Division, Beckman Instruments Inc., Calif., U.S.A.) for one hour.

Preparation of rabbit hyperimmune sera. Antigens used were a) ten-fold concentrates of preparations of adenovirus prototypes 5, 6, 9 and 11, b) soluble complete hemagglutinin (HA) of adenovirus type 11 purified by zonal centrifugation (Norrby, 1968a), c) adenovirus type 5 material propagated on primary rabbit kidney cells maintained in Parker's medium 199 with 2% rabbit serum from the animal to be immunized, and d) intact virions of adenovirus type 9 which had been subjected to two consecutive isopycnic