The Structure of Group II Adenoviruses

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SUMMARY

Adenovirus types 13, 15, and 19 and their soluble antigens were studied by electron microscopy, applying the negative staining technique to purified material. Virus particles showed projections of 160 to 190 Å length with a final knob arising from the 12 vertices of the icosahedron. Incomplete particles were also seen. The group-specific antigen consisted of free ('hexon') capsomeres. The type-specific antigen, which showed the phenomenon of haemagglutination in the presence of a heterologous immune serum, had the appearance of fibres with final knobs ('fibre antigen'). The soluble haemagglutinin appeared as a star with a diameter of 450 to 600 Å. It was composed of presumably 12 dumb-bell-shaped subunits, arranged in a regular fashion. The virus types studied were morphologically more closely related to type 3 (group I) than to type 5 (group III).

INTRODUCTION

Group II human adenoviruses have been defined as comprising the serotypes which agglutinate rat erythrocytes with a complete haemagglutination pattern (Rosen, 1960). This virus group and their haemagglutinins have been investigated (Bauer, Wigand & Adam, 1964; Wigand & Stöhr, 1965), but the structural basis for the observed biological and serological phenomena have remained obscure. In the present report viruses of group II and their soluble antigens were studied by electron microscopy. Some other physical data on these virus components are also reported.

An informative structure model of adenovirus type 5 (of group III) has been proposed by Valentine & Pereira (1965). This model, however attractive, may not be valid for all other adenovirus types since remarkable differences in biological, serological, and biochemical properties between adenoviruses of the various groups and of their soluble antigens have been found (for a review see Wigand, 1967). Recently Norrby (1966a) studied type 3 (of group I) and found some differences in the detail of the structure of the virion, compared with type 5 (group III). Furthermore, the structure of the soluble haemagglutinin of type 3 had no counterpart in type 5.

Evidence is presented here that the structure of group II adenoviruses is similar to that of group I. Nevertheless, differences were observed to both groups I and III.
METHODS

Viruses. Prototype strains of adenovirus 13, 15, and 19 representative of group II were grown in serum-free HeLa cell cultures. Since it was found that the culture fluid generally contained only small amounts of viral antigens, only the cells were harvested one day after full development of the cytopathic effect. The viral antigens were liberated by freezing and thawing the cells three times in 0.05 vol. of saline. The cell debris was removed by centrifuge and the supernatant fluid used as starting material.

Separation of antigens. As reported earlier (Wigand et al. 1966), a combination of different methods is needed to separate the infective virus, the soluble haemagglutinin and the group-specific complement-fixing (CF) antigen from one another. In addition, a soluble type-specific component could be separated from the other antigens by chromatography on DEAE-cellulose at pH 5.2; it passed through the column without being adsorbed. The crude virus suspension was dialysed against 0.01 M-phosphate buffer pH 5.2 and chromatographed at this pH. Fractions containing all other viral activities except the type-specific antigen were concentrated by ultrafiltration. Thereafter they were adjusted with solid CsCl to an optical refraction of $n_0 = 1.365$ and centrifuged in an SW 39 rotor (Spinco L centrifuge) for 20 hr at 35,000 rev./min. Two bands were visible in the tube, the lower containing the infectious virus, the upper incomplete particles and other viral antigens. This second band could be separated into a lower portion containing the bulk of incomplete particles, and an upper portion containing the group-specific antigen and the soluble haemagglutinin. The infectious virus was recentrifuged in CsCl and tested biologically by haemagglutination with rat erythrocytes or by infectivity. Soluble haemagglutinin, group-specific antigen and incomplete particles were separated by layering the serologically active material from the CsCl gradient on the top of a preformed sucrose gradient (45 to 10 %) and centrifuging for 4 hr at 35,000 rev./min. Fractions from density gradients were collected through a hole punctured in the bottom of the tubes. The purified materials to be examined by electron microscopy were dialysed against 0.14 M-ammonium acetate and tested for activity and purity by appropriate biological methods.

Biological tests. Virus titration, haemagglutination (HA), and complement-fixation were performed as described earlier (Bauer & Wigand, 1963; Wigand et al. 1966). Human convalescent serum of one of us (H.G.) was used for determination of the group-specific antigen. Type-specific antisera were obtained from rabbits immunized with the homologous virus type prepared in serum-free human thyroid cell cultures. These sera did not react with HeLa cell material or with constituents of the medium.

Electron microscopy. Viral antigens, after dialysis against ammonium acetate, were mixed with an equal amount of 2 % (w/v) phosphotungstic acid (adjusted with KOH to a pH of 7.2), placed on formvar-coated grids, drained after 30 sec. and examined by a Siemens Elmiskop I at a magnification of × 40,000.

Determination of sedimentation rates. One half ml. of purified viral antigen was layered on a sucrose gradient (30 to 15 % in 0.1 M-NaCl, 0.01 M-tris + HCl buffered pH 7.2, 0.001 M-EDTA) and centrifuged in an SW25 rotor at 15°. In the experiment with group-specific CF antigen, tobacco mosaic virus RNA was centrifuged as reference in a second tube. For the type-specific antigen, bovine serum albumin and IgG (7 S) HA-inhibiting antibody from the rabbit was used for reference. In the experiment with the haemagglutinin, the reference was ME virus labelled with [3H]uridine (Hausen &
Schäfer, 1962). After centrifugation, 1 ml. fractions were collected from the bottoms of the tubes and the respective activities were measured in the different fractions by appropriate tests, i.e. haemagglutinating and serological activity with antigens, u.v. adsorption at 260 m\(\mu\) for tobacco mosaic virus RNA, protein determination according to Lowry et al. (1951) for bovine albumin, HA-inhibition against adenovirus 19 for HA-inhibiting antibodies, and radioactivity for ME virus.

RESULTS

The structure of the viral components was identical for the three virus types studied.

Complete virus particles

The structure of virus particles, as shown by the negative-staining technique, corresponded to the icosahedron model first described by Horne et al. (1959) (Pl. 1, fig. 1). The capsomeres were regularly arranged, the number in each edge (six) corresponding to a total number of 252 capsomeres. Projections were seen extending from the vertices of some particles depending on the thickness of the phosphotungstate (Pl. 1, fig. 1a, b). The average length of these projections was 160 to 190 Å, including the final knob with a diameter of 30 to 40 Å.

![Sucrose density gradient diagram for estimation of the sedimentation rate of group CF antigen](image)

Fig. 1. Sucrose density gradient diagram for estimation of the sedimentation rate of group CF antigen. Reference material: tobacco mosaic virus RNA, 29S. O—O, adeno-19 CF-antigen; ×—×, TMV-RNA.

Incomplete particles

Empty particles corresponded in size and overall structure to complete particles. The phosphotungstate has apparently entered the particles; the capsomeres were not clearly visible (Pl. 1, fig. 2). Projections arising from the vertices were occasionally seen.

Group-specific antigen

This material consisted entirely of free capsomeres (Pl. 1, fig. 3), which appeared as hollow particles of 75 to 80 Å. The circumference of the hole appeared to be composed of several (possibly six) morphological subunits. The sedimentation rate was estimated as described under methods (Fig. 1). The calculated value of 11·5 S corresponds with
that found by Köhler (1965) for the group-specific antigen of type 2, which was determined in the analytical ultracentrifuge.

**Soluble haemagglutinin**

The virus-free haemagglutinin had a star-like appearance (Pl. 1, fig. 4, 5). It consisted of a number of capsomere-like granules and projections arising radially from them, ending in a terminal knob. These substructures appeared to be regularly arranged. The model of 12 dumb-bell-shaped morphological subunits arranged in a manner of 5:3:2 symmetry (Pl. 1, fig. 5c, e) is compatible with the appearance of the particles. Each subunit presumably consists of a vertex capsomere + projection, similar in length to that on the virus particle. Minor differences in appearance of the particles may have been due to different orientation and superposition of the capsomeres and, in some instances, to degradation (Pl. 1, fig. 4a, d). The sedimentation rate of the soluble haemagglutinin was approximately 60 S (Fig. 2).

![Figure 2](image-url)

**Type-specific antigen**

Difficulties were encountered in studying this material because it was present in relatively small amounts and because of its small particle size. The antigen preparations mainly showed fine fibres, ending in a knob (Pl. 2, fig. 6, 7), which in some cases appeared hollow. The dimensions were similar to those of the projections of the virion and of the soluble haemagglutinin, i.e. 150 to 190 Å in length. Dumb-bell-shaped particles with an end capsomere could not be identified with certainty. Structures of this kind were, however, occasionally seen in preparations of spontaneously disintegrating particles (Pl. 2, fig. 8).

The sedimentation rate of the type-specific antigen was approximately 62 S. Köhler (1965) found 55 S for the type-specific (fibre) antigen of type 2. It is of interest that the type-specific antigen of group II viruses shows the phenomenon of HA in the presence of a heterologous adenovirus immune serum (indirect HA). This was found originally only in group III adenoviruses (Rosen, 1960), but recently also in type 3 of group I (Norrby, 1966b).
DISCUSSION

The structural model proposed by Valentine & Pereira (1965) for adenovirus 5, which consists of 240 ‘hexon’ (Ginsberg et al. 1966) capsomeres and 12 vertex capsomeres with projections (‘pentons’), appears to be valid in general terms also for adenoviruses of group II. There is, however, a marked difference in the length of the projections between the three groups, which (including the terminal knob) measure 150 to 190 Å in group II viruses, up to 240 Å for type 5 (group III, Valentine & Pereira, 1965), and 80 to 110 Å for type 3 (group I, Norrby, 1966a). The type-specific antigen and the projections of the soluble haemagglutinin have lengths similar to those of the projections of the virus particle. Apparently either the projections or projections + vertex capsomeres of the complete particle carry its type-specific haemagglutinating activity. The projections arising from soluble haemagglutinin are seen more easily than those arising from the virus particles. This may be due to the greater sizes of the complete particles which render the thickness of the phosphotungstate more critical.

The type-specific antigen appears to be analogous to, but shorter in length than, the C or fibre antigen of type 5 (Wilcox, Ginsberg & Anderson, 1963; Valentine & Pereira, 1965). This analogy is strengthened by the fact that antigens of type 5 as well as of group II viruses agglutinate rat erythrocytes in the presence of a heterologous immune serum. These indirect haemagglutinins may have one type-specific determinant at one end, possibly the knob, reacting with the erythrocyte surface, and another determinant of broader specificity at the other end, which reacts with bivalent antibody of the heterologous adenovirus immune serum; that in turn leads to HA. This mechanism was suggested by Pereira & de Figueiredo (1962) for group III viruses.

We found no cytotoxic activity of the fibre antigen for the types studied in HeLa cells. This was not unexpected, since viruses of group II appear to be free of toxic activity. Furthermore, in type 5 the toxin is associated with B or penton antigen (Valentine & Pereira, 1965); penton-like structures were not found as soluble antigen in group II viruses, although they were seen in fragmented virus particles (Pl. 2, fig. 8).

The soluble haemagglutinin appears to consist of a regular array of twelve morphological subunits arranged in accordance with icosahedral symmetry. Each capsomere-like base has five neighbours (Pl. 1, fig. 5), which is also true for the vertex capsomeres of the complete particle. While no similar particles have been found in type 2 (Köhler, 1962) or type 5, the structure model proposed by Norrby (1966a) for the haemagglutinin of type 3 (group I) is similar. If the correctness of the model is established, the term *dodecon* (as analogous to hexon and penton, Ginsberg et al. 1966) for this structure unit would seem appropriate. It is possible that similar structures also exist in group III adenoviruses, but being devoid of haemagglutinating activity with known erythrocytes they may have escaped detection. Certainly the soluble haemagglutinins of group II cannot be incorporated as a whole into virus particles. They appear to be formed as a by-product of virus synthesis, simultaneously with the infective particles (Wigand & Wunn, 1967). The above observations fit our earlier findings that the haemagglutinins of group III viruses differed in several properties from those of group I and II (Wigand & Pauer, 1962; Bauer & Wigand, 1963).

While the morphological substrate of the various haemagglutinating particles (complete particle, incomplete particle, soluble haemagglutinin, type-specific antigen)
appear to be similar (i.e. fibre + knob), these haemagglutinins are by no means identical in their biological and serological properties. Thus the virus-associated haemagglutinin has, at least in type 15, a broader serological reactivity than the soluble haemagglutinin (Wigand & Wunn, 1966). However, the indirect haemagglutinin appears to be similar to the soluble haemagglutinin in its serological relationship to other types (unpublished results). Furthermore, the host range of agglutinable erythrocytes is identical for complete and incomplete particles, but different for the soluble haemagglutinin in all three virus types studied (Table 1; Wigand & Stöhr, 1965). At present it is impossible to account for these differences on a structural basis except by plain speculation.

![Table I. Some properties of adenovirus components](image)

The particles with group specificity are free hexon capsomeres, as found for type 5 (Wilcox et al. 1963; Valentine & Pereira, 1965) and 3 (Norrby, 1966a). Despite the group reactivity, the hexon antigens of different virus types are not identical. Thus elution profiles from DEAE-cellulose are different for the group antigens in group II and III (Gelderblom, Wigand & Bauer, 1965). Furthermore, the group-specific antigen seems to have a type-specific determinant as well, since type-specific neutralizing antibodies can be elicited by immunizing animals with purified hexon antigens. This was found for type 5 (Wilcox & Ginsberg, 1963), as well as for type 19 (unpublished results).

The physical data of the various virus components are summarized in Table 1. The sedimentation rate for the group-specific antigen and the soluble haemagglutinin is somewhat higher than the estimated minimal values reported earlier (Wigand et al. 1966). With respect to the buoyant density in CsCl, it is understandable that empty particles and free capsomeres have a similar density of 1.31.

After the structural elements of the components of adenoviruses of group II have been elucidated, the serological relationships between these antigens are of obvious interest. This will be the subject of another report.

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REFERENCES


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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Complete virus particles. a to c: type 13, d: type 19.
Fig. 2. Incomplete virus particles (type 15).
Fig. 3. Complement-fixing group antigen (free capsomeres) (type 13).
Fig. 4. Soluble haemagglutinin. a, b: type 13; c, d: type 19.
Fig. 5. Soluble haemagglutinin. a, b: type 13; c, e: model, d: type 19.

PLATE 2

Fig. 6. Type-specific or fibre antigen (type 19) (arrows).
Fig. 7. Type-specific antigen (type 19) (arrows).
Fig. 8 Disintegrating virus particles (type 19) (Arrow: dumb-bell-shaped elements, consisting of vertex capsomere + projection + knob.)