Incomplete Virus Particles of Adenovirus Type 16

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SUMMARY

Haemagglutinating populations of virus particles of adenovirus type 16 (I–VIII) were separated by isopycnic centrifuging in CsCl. One of these populations (V) contained complete virus particles. Fresh preparations of virus particles from all populations studied had a morphology indistinguishable from that of complete virus particles.

The five lightest populations were shown to contain [3H]-thymidine and to be infective; dose–response relationships indicated the occurrence of a multiplicity-dependent infectivity of the incomplete virus particles. SDS-polyacrylamide gel electrophoresis of incomplete virus particles of the lightest population demonstrated that they were deficient in three polypeptides, but contained three other polypeptides which were not detectable in complete particles. One characteristic polypeptide of incomplete particles and another characteristic polypeptide of complete particles displayed high ratios of arginine over threonine.

INTRODUCTION

Defective or incomplete virus populations, i.e. particles containing less nucleic acid than complete particles, have been found in all the major groups of animal RNA viruses (Huang & Baltimore, 1970). Some animal DNA viruses such as polyoma (Crawford, Crawford & Watson, 1962; Blackstein, Stanners & Farmilo, 1969) and SV 40 (Yoshiike & Furuno, 1969) have also been shown to contain populations of incomplete virus particles. Adenovirus particles (top components or empty capsids) having lower buoyant densities than complete particles have been demonstrated previously (Köhler, 1962; Norrby, Grönberg & Magnusson, 1964; Smith, 1965; Shimojo et al. 1967; Wadell & Skaaret, 1967; Maizel, White & Scharff, 1968). These virus particles, which have been reported to lack (Maizel et al. 1968) and to contain DNA (Burlingham & Doerfler, 1969; Mak, 1971; Prage, Höglund & Philipson, 1972), display a higher buoyant density than the structural proteins which form the virus capsid (Wadell & Skaaret, 1967).

Our main intention was to study the composition and biological activity of incomplete virus of an oncogenic adenovirus type. Providing the occurrence of virus specified DNA in incomplete virus can be established, an infection with incomplete virus at low input multiplicities could represent an enhanced risk of transformation, whereas a possibility exists that complementation could occur between incomplete virus particles after infection at high input multiplicities. Furthermore, elucidation of the polypeptide composition of incomplete virus could be important in studies of the synthesis and assembly of adenovirus.

Adenovirus type 16 (Ad 16) – a member of Rosen’s subgroup I – was selected for the
present study since the proportion of incomplete Ad 16 virus was considerably higher than
in preparations of the more frequently studied virus types belonging to Rosen's subgroup III
(Maizel et al. 1968). In this paper we describe the occurrence of eight populations of virus
particles of adenovirus type 16 which have distinct buoyant densities. It will be shown that,
after extensive purification, the four populations of particles with densities lower than that of
complete particles appear on electronmicrographs as intact virus particles and contain DNA
and characteristic polypeptides some of which cannot be detected in complete virus particles.
In addition, each population has been found to carry a certain degree of infectivity. Some of
this information has been presented in a preliminary report (Wadell & Hammarskjöld,
1970).

METHODS

Virus and cell cultures. The prototype strain (Ch 79 isolated by Dr S. Chang) of adenovirus
type 16 was passaged 12 times prior to arrival and 3 times in this laboratory. Virus was
propagated in stationary or roller cultures of KB cells (Flow Laboratories, Irvine, Scotland)
which were routinely discarded after 20 passages in our laboratory and free of detectable
mycoplasma. Adenovirus-associated virus particles have not been detected after screenings
of fractions obtained by centrifuging on discontinuous CsCl gradients (see below) by electron
microscopy. The cells were maintained in Eagle's minimal essential medium (MEM)
(Eagle, 1959) containing twice the normal amount of amino acids and vitamins (Eagle's
MEM x 2) and supplied with 2 % calf serum. Roller cultures of KB cells to be used in
preparations of isotope-labelled virus were grown out in the above-mentioned medium
containing 5 % foetal bovine serum (Flow Laboratories). KB cells were infected at an input
multiplicity of 1·5 to 5 TCD50/cell with virus infected cells, which had been frozen and thawed
once in their maintenance medium (crude virus fluid). Three to four days later the infected
cells were harvested and sedimented at 200 g for 15 min. Pellets of about 10^9 cells were
resuspended in 30 ml of 0·02 M-tris buffer, pH 9·0, containing 0·2 % sodium deoxycholate
(E. Merck AG, Darmstadt, Germany). After gentle agitation for 1 h at 22 °C cell debris
was removed by two sequential centrifugings at 200 g for 15 min.

Radioactive labelling of virus. [3H]-thymidine: 3 h after seeding with crude virus fluid, the
medium in each bottle (containing 6 to 7 x 10^6 cells) was changed to 25 ml containing 0·1
mCi [3H]-thymidine (methyl-T, specific activity exceeding 10 Ci/mol, obtained from the
Radiochemical Centre, Amersham, England). Another 25 ml of maintenance medium was
added 1 day after inoculation of cultures.

[3H]-arginine and [14C]-threonine: the amount of arginine and threonine in Eagle's
medium was considerably reduced in order to obtain an efficient labelling of virus peptides.
Under this condition of partial deprivation of arginine and threonine the amount of virus
particles synthesized was one fourth to one third of the amount obtained with Eagle's
MEM x 2. The relative proportion between complete and incomplete virus particles was not
significantly altered. Unfractionated virus at an input multiplicity of 3 f.f.u./ml was allowed
to adsorb to cells in roller cultures for 2 h at 37 °C. The medium containing virus was then
discarded and 30 ml of Eagle's medium with the amounts of threonine and arginine reduced
to 0·08 and 0·06 mm, respectively was added. This medium was supplemented with 0·5 %
calf serum, which had been dialysed against phosphate-buffered physiological saline (PBS),
0·067 M, pH 7·2. At twenty-four h post-infection this medium was discarded and 30 ml of
Eagle's medium which contained 0·025 mm-threonine and 0·015 mm-arginine in addition to
30 μCi of [3H]-threonine (180 mCi/m-mol) and 30 μCi of [3H]-arginine (25 Ci/m-mol) was
added. Cells were harvested after 68 h and treated as described above.
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Infectivity assays. Purified virus particles in CsCl were diluted 1:5 in Eagle's MEM containing 0.2% bovine serum albumin and dialysed against Eagles MEM for 4 h or overnight at 4 °C. The infectivity was then assayed by two different techniques.

(A) Titration of virus in tubes containing human diploid fibroblasts maintained in Eagle's MEM supplemented with 2% calf serum. Tenfold serial dilutions of virus, with 4 to 5 tubes for each dilution, were used. Final readings of the tubes were taken after 3 weeks in a rolling state. The 50% tissue culture infectious dose (TCID₅₀) was calculated according to Reed & Muench (1938).

(B) Fluorescent focus assay of single infected cells (Philipson, 1961). Confluent monolayers of human diploid fibroblasts or KB cells grown on coverslips in Leighton tubes were used. Twofold dilutions of virus was adsorbed in 0.4 ml samples to cells in 2 or 3 tubes per dilution. During adsorption for 2 h the tubes were rocked by a 'Rocker platform' (Belco Glass Inc., Vineland, New Jersey, U.S.A.) the tubes were rinsed once with Eagle's MEM after adsorption and Eagle's MEM supplemented with 2% calf serum was added. Infected fibroblasts and KB cells were fixed in acetone after 46 h. The fixed cells were incubated with homotypic anti-virus serum for 30 min at 37 °C. After extensive rinsing the coverslips were incubated for 30 min at 37 °C with fluorothioisocyanate conjugated sheep anti rabbit serum (weight F/P ratio of about 5 × 10⁶). After mounting, the cultures were examined in a Leitz Dialux microscope using a 16 × objective and 10 × ocular.

Haemagglutination (HA) tests. Purified virus particles in CsCl were diluted in tubes containing PBS, and titrated in serial twofold dilutions in 0.4 ml vol. 0.2 ml of a 0.5% suspension of green monkey (Cercopithecus aethiops) erythrocytes was added to each tube followed by incubation at 37 °C for 2 h. The initial dilution was selected so that no more than seven twofold dilution steps were required to reach the last tube in which there was a clear-cut agglutination. This tube was considered to contain 1 HA unit (H.A.U.). Adeno-virus type 16 contains the following haemagglutinins: complete and incomplete virus particles, dimers ofpentons and fibres (Norrby & Skaaret, 1968). The latter (soluble haemagglutinins) are efficiently separated from virus particles by centrifuging in a discontinuous CsCl gradient (see below). Three purified preparations of incomplete virus particles (population I) and complete particles (population V) contained on the average 87 and 94 H.A.U./µg, respectively. No significant difference in haemagglutinating activity was thus noted between complete and incomplete virus particles.

Separation of virus particles. Ten ml of clarified virus material was layered on to discontinuous CsCl (American Potash Corporation, West Chicago, Ill.) gradients and centrifuged for 90 min at 25,500 rev/min and 4 °C (SW 25.1, Spinco Division, Beckman Instruments, Calif.). The discontinuous gradients consisted of layers of CsCl dissolved in 0.04 M-tris-HCl, pH 8.0, of the following composition: 2 ml 1.45 g/ml; 10 ml, 1.32 g/ml; 10 ml, 1.25 g/ml. After centrifuging the material was harvested via the bottom of the tube in fractions of equal size.

Equilibrium sedimentation of virus particles in CsCl. Sedimentations were performed in fixed-angle rotors R 40 and R 50 (Spinco). Virus preparations were centrifuged for 40 to 80 h at 4 °C at a speed chosen to give a gradient of suitable steepness. Thirty to forty equally sized fractions were collected. The density was determined on the day of harvest by weighing 100 µl samples in a calibrated constriction pipette.

Determination of radioactivity. Assay of radioactive material in liquid samples: 10 µl samples and 10 ml liquid scintillator (composed of 7 g 2,5-diphenyloxazole, 0.6 g 1,4-tris-2 (4-methyl-5-phenyloxazolyle)-benzene, 150 g naphthalene in 300 ml ethylene glycol monomethyl ether and 550 ml toluene) per sample were added to scintillator vessels. Assay of
radioactivity in slices of polyacrylamide gel: frozen gel slices were placed in scintillator vessels and 0.6 ml of 3% piperidine solution in distilled water was added. After incubation overnight, 10 ml of Insta-gel (Packard Instruments, Downers Grove, Ill., U.S.A.) was added. The samples were counted for 4 or 10 min at constant efficiency in an Intertechnique SLAC 40/4 liquid scintillation spectrometer.

Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. Gel consisting of 7.5% (w/v) polyacrylamide and 0.20% N,N'-bis-methylene acrylamide in gel buffer (7.8 g NaH₂PO₄·H₂O, 38.6 g Na₂HPO₄·7H₂O, 2 g SDS per l (Kebo AB, Stockholm), 0.075% ammonium persulphate (Merck AG) and 0.075% N,N',N'-tetramethylethlenediamine (Fluka AG, Buchs, SG, Switzerland) as described by Weber & Osborn (1969), were found to be suitable for analysis of virus polypeptides. A linear correlation was obtained in the range of mol. wts. of 11700 to 130000, between the mobility and the logarithm of mol. wts. of reference polypeptides. Acrylamide and N,N'-bismethylene acrylamide (Eastman Organic Chemicals, Rochester, New York) were recrystallized from chloroform and acetone, respectively. A few drops of water were layered on top of the gel, which was 80 mm long and had a diam. of 4 mm, before hardening. Virus proteins were precipitated with 4 vol. of acetone and left overnight at −20 °C. The samples were then denatured and reduced in 0.01 M-sodium phosphate buffer containing 1% SDS and 1% β-mercaptoethanol, pH 7.0, and heated to 100 °C for 1 to 2 min. Dissolved proteins containing 10% glycerol and a trace of bromphenol blue marker (Merck AG) were layered on top of each gel. The two buffer compartments of the electrophoresis apparatus were then filled with gel buffer, diluted 3:1 with water. The electrophoresis was carried out at a constant current of 8 mA per gel for 2 to 4 h. The gels were stained for 12 h in a solution of 0.25% Coomassie Brilliant Blue R (Edw. Gurr Ltd., London) in a mixture of 50% methanol and glacial acetic acid (9:1). Destaining of gels was performed in a solution containing ethanol, glacial acetic acid and water (25:8:65) (O. Vesterberg, personal communication). Gels containing radioactively labelled polypeptides were frozen at −70 °C and then sliced into equally sized 1 mm discs.

Protein determination. The method of Lowry et al. (1951) was used with bovine serum albumin as a standard.

Estimation of mol. wts. The method described by Shapiro, Vinuela & Maizel (1967) and Weber & Osborn (1969) was used. Polypeptides from the following proteins were used as references: β-galactosidase mol. wt. 130000 (Ullman et al. 1968) provided by Dr Franco Celada, Karolinska Institutet; bovine serum albumin and alcohol dehydrogenase (horse liver) (Sigma) mol. wts. 68000 and 41000, respectively (Castellino & Barker, 1968); trypsin (Merck), cytochrome c (Schwartz/Mann, Orangeburg, New York) mol. wts. 23000 and 11700, respectively (Weber & Osborn, 1969) and ribonuclease A (Worthington, Biochemical Co., Freehold, New Jersey) mol. wt. 13700 (Smyth, Stein & Moore, 1963).

Electron microscopy. Virus particles purified by equilibrium sedimentation were placed on a carbon-coated copper grid and rinsed after 30 s with a solution of 2% sodium silicotungstate in distilled water. The specimens were examined as previously described (Wadell, Norrby & Skaaret, 1969).

RESULTS

Identification of different populations of adenovirus particles

Stock virus material derived from 5 x 10⁸ deoxycholate-disrupted cells was submitted to centrifuging on a discontinuous CsCl gradient to obtain virus particles clearly separated from subcellular components and soluble virus subunits. After centrifuging, virus particles formed two broad bands in the lower part of the discontinuous CsCl gradient correspond-
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Fig. 1. Separation of virus particles from subcellular components and soluble virus subunits. The relative amounts of unfractionated virus material and CsCl of different densities layered to form a discontinuous gradient is shown on the top of the diagram. Distribution of haemagglutinating activity (○—○) expressed as H.A.U. per 0.4 ml; [3H]-thymidine labelled material (●—●) expressed as ct/min/0.025 ml and $E_{280}$ (---) after centrifuging in a swing-out rotor (SW 25.1, 22500 rev/min, 90 min) is demonstrated.

The virus particles were subjected to equilibrium density sedimentation in CsCl using a fixed-angle rotor, since this separation procedure has been reported to give good resolution of components which display minor differences in buoyant density (Flamm, Birnstiel & Walker, 1969). The major portion of the particles in the two broad bands obtained after centrifuging in the discontinuous CsCl gradient were centrifuged separately (gradients (a) and (c), Fig. 2) in parallel with the particles recovered from the region between the two major bands (gradient (b), Fig. 2).

After centrifuging five bands were observed in gradient b corresponding to different virus particle populations, which have been denoted populations I to V in order of increasing buoyant density (Table 1). Gradients (c) and (a) contained only one band of virus particles corresponding to populations I and V (complete particles), respectively.

The extent of contamination of incomplete virus particles by cellular polypeptides was studied. Uninfected KB cells, kept in maintenance medium, were labelled for 68 h with [3H]-threonine to a specific radioactivity of 1640 ct/min/μg and mixed with equal amounts of...
Fig. 2. Identification of virus particle populations I to V after equilibrium sedimentation in CsCl with an average density of 1.32 g/ml using a fixed-angle rotor (R 40, 32 000 rev/min, 80 h, 4 °C). Tubes (a), (b) and (c) contain the virus particles recovered at high (1.345 g/ml), intermediate (1.31 to 1.34 g/ml) and low density (1.30 g/ml) after the initial separation on a discontinuous CsCl gradient (see Methods).

Table 1. **Buoyant density, [H]-thymidine ct/min/H.A.U. × 10⁻³ and TCD₉₀/H.A.U. ratios of different particle populations of adenovirus type 16**

<table>
<thead>
<tr>
<th>Population</th>
<th>Buoyant density (g/ml)</th>
<th>[H]-thymidine (ct/min; H.A.U. × 10⁻³) *</th>
<th>TCD₉₀/H.A.U. †</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.298 to 1.307</td>
<td>0.5-2.0</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>1.310 to 1.323</td>
<td>6-15</td>
<td>7-45</td>
</tr>
<tr>
<td>III</td>
<td>1.326</td>
<td>25</td>
<td>450</td>
</tr>
<tr>
<td>IV</td>
<td>1.330 to 1.338</td>
<td>27</td>
<td>1000</td>
</tr>
<tr>
<td>V</td>
<td>1.344</td>
<td>40</td>
<td>22000</td>
</tr>
<tr>
<td>VI</td>
<td>1.349 to 1.353</td>
<td>N.T. ‡</td>
<td>N.T.</td>
</tr>
<tr>
<td>VII</td>
<td>1.363</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>VIII</td>
<td>1.38 to 1.39</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
</tbody>
</table>

* The ratios of radioactivity per 5 µl over haemagglutinating activity per 0.4 ml were obtained for two preparations of isolated virus particle populations.

† Ratio of TCD₉₀/0.1 ml over H.A.U./0.4 ml calculated on two or three preparations of isolated virus particle populations.

‡ Not tested on isolated populations of virus particles.
Table 2. Contamination of virus particle populations I and V with host proteins

<table>
<thead>
<tr>
<th>Virus particle population</th>
<th>Mixture of uninfected and infected cells</th>
<th>Discontinuous CsCl gradient sedimentation</th>
<th>Second equilibrium sedimentation</th>
<th>Final contamination of virus particles†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>$2 \times 10^7$</td>
<td>270,000</td>
<td>330</td>
<td>0.02 %</td>
</tr>
<tr>
<td>V</td>
<td>$2 \times 10^7$</td>
<td>25,500</td>
<td>35</td>
<td>0.0006 %</td>
</tr>
</tbody>
</table>

* Equal amounts of uninfected and infected cells were mixed and extracted with sodium deoxycholate.† The amount of protein in purified virus particle populations was determined according to Lowry et al. (1951) and the percentage of host cell contamination was calculated from the specific radioactivity of uninfected host cells (1640 cts/min/μg).

In order to establish whether virus particles banding at densities lower than complete particles contained DNA, [3H]-thymidine-labelled virus preparations were separated according to the method described in the previous section. Each virus particle population was isolated by two consecutive equilibrium sedimentations and thymidine-labelled DNA was found to be associated with virus particle populations I to V. The relative proportion of radioactivity as compared to haemagglutinating activity decreased in virus particles banding at lower buoyant density (Table 1). The DNA associated with incomplete virus particles has been found to be resistant to treatment with DNase (M-L. Hammarskjöld, G. Wadell & E. Norrby, unpublished observations).

**Incorporation of [3H]-thymidine into incomplete virus particles**

In order to establish whether virus particles banding at densities lower than complete particles contained DNA, [3H]-thymidine-labelled virus preparations were separated according to the method described in the previous section. Each virus particle population was isolated by two consecutive equilibrium sedimentations and thymidine-labelled DNA was found to be associated with virus particle populations I to V. The relative proportion of radioactivity as compared to haemagglutinating activity decreased in virus particles banding at lower buoyant density (Table 1). The DNA associated with incomplete virus particles has been found to be resistant to treatment with DNase (M-L. Hammarskjöld, G. Wadell & E. Norrby, unpublished observations).

**Determination of the infectivity of different populations of virus particles**

The results presented in the previous section seemed to indicate the occurrence of DNA in virus particle populations I to V, and each of the five populations was isolated in order to determine whether they carried infectious activity. After careful separation of populations I to III by three consecutive isopycnic sedimentations no detectable (Fig. 3c), or only minor, peaks (Fig. 3a, b) of infectivity were found in the density region where complete particles could be expected to appear. The appearance of peaks of infectivity but no haemagglutinating activity at the buoyant density of complete particles is probably a function of the pronounced difference in sensitivity in the detection of infectivity and haemagglutination. The major part of the infectivity after the final separation of the three virus particle popula-
Fig. 3. Distribution of infectious and haemagglutinating activities after separation of the various virus particle populations of adenovirus type 16. Three consecutive equilibrium sedimentations in fixed-angle rotors were used in the isolation of populations I to IV: (a) Population I (rotor 50, 29000 rev/min, 60 h); (b) population II (rotor 40, 32000 rev/min, 60 h); (c) population III (rotor 40, 32000 rev/min, 60 h); (d) population IV (rotor 50, 29000 rev/min, 60 h). (e) Demonstrates the biological activities of complete particles (population V) and the high-density virus particle populations after the second isopycnic sedimentation (rotor 40, 32000 rev/min, 60 h). The arrow indicates the buoyant density of intact virus. The average density was 1.32 g/ml in gradients (a) to (c) and 1.35 g/ml in gradients (d) and (e). ● ● H.A.U.; ○ ○ infectious virus.

The TCD₅₀/H.A.U. ratio noted for populations I-IV ranged from 2 to 1000 whereas that for complete particles (population V) was 22000 (Table 1, Fig. 3). No significant difference
Fig. 4. Dose–response relations determined by fluorescent focus titrations of incomplete virus particle populations I to IV and intact virus particles V in human diploid fibroblasts. Twofold dilutions of six preparations of population I (○), two preparations of populations II, III-IV and V (●), and separate tenfold dilutions of six preparations of population V (□) are presented. Bar equals one s.d.

in haemagglutinating activity per unit protein was noted between complete and incomplete virus particles (see Methods). The number of physically incomplete virus particles per infectious unit thus was considerably higher than the number of physically complete particles corresponding to one infectious unit. Dose–response experiments were therefore performed to determine whether the infectivity of the four incomplete populations of virus particles was multiplicity dependent. By relating the number of single infected fluorescent cells to virus diluted in twofold steps a characteristic one-hit dose–response curve was obtained after titration of intact particles (population V). Titration of infectivity versus dilution of virus particles of populations I to IV resulted in curves with steeper slopes than that obtained for intact virus. Titrations of different preparations of incomplete virus showed larger variations than assays of complete virus (Fig 4). Varying amounts of aggregates of virus particles between different preparations could represent one explanation of this observation. To determine the extent of possible aggregation of virus particles, zonal sedimentation of [3H]-thymidine-labelled virus particles from populations I to V obtained after one isopycnic sedimentation were performed on linear 15 to 35 % (w/v) sucrose gradients after dialysis against 5 % (w/v) sucrose in 0.05 M-tris, pH 8.0. Three to six % of the radioactivity in these virus particle populations sedimented as aggregates of two or more virus particles.

During the investigation of particle populations I to V, haemagglutinating virus particles displaying a higher buoyant density than complete particles were observed. The analysis of these populations (VI-VIII) has not yet been completed.
Morphology of different populations of virus particles

The ultrastructure of the capsids of complete particles and of fresh preparations of the seven incomplete virus particle populations could not be distinguished. However, virus particles from populations I to IV seem to be susceptible to stress during the staining procedure, since micrographs on which nearly all particles appeared as disrupted shells as well as micrographs on which the majority of the particles appeared as intact capsids (Fig. 5a) could be obtained from the same preparations of incomplete virus. In order to visualize possible internal components in the virus particles of population I, these particles and control complete particles (population V) were treated with 5% (v/v) diethylpyrocarbonate ('Baycovin', Bayer Farbenfabrik AG, Leverkusen, Germany) in 0.2 M-sodium acetate, pH 5.8, for 16 h at 22 °C. After this treatment the sodium silicotungstate (SST) could penetrate the capsid of most particles through visible cracks. Virus treated in this way appeared to be almost filled by internal components (Fig. 5d). The morphology of treated virus particles from population I (Fig. 5b) differed from that of treated complete particles which either indicates that comparatively more SST penetrates into population I particles or that these particles were more easily deformed than complete particles forming impressions which can be filled by SST as suggested by Crawford et al. (1962) in studies of polyoma virus particles.
Fig. 6. Stained gels showing the electrophoresis of the proteins of virus particle populations I to V of adenovirus type 16 in neutral SDS containing polyacrylamide (7.5\%, w/v).

Mol. wt. determination of polypeptides of complete and incomplete particles of adenovirus type 16

Eight bands of virus-specific polypeptides were observed after electrophoresis as described under Methods (Fig. 6). Their relative mobility was indicated both by the position of stained material and by the peak of radioactivity (Fig. 7) and the following mol. wts. were determined: (1) 124000 ± 5000, (2) 79000 ± 2000, (3) 60000 ± 2000, (4) 54000 ± 2000, (5) 37000 ± 2000, (6) 27500 ± 1500, (7) 26000 ± 2000, and (8) 13000 ± 1000.

Virus particle populations I to V all displayed bands (1), (2) and (8), the latter in small amounts. The remaining polypeptides were distributed in the following characteristic patterns in virus particle populations I to V: population I – polypeptides 3–6; population II – polypeptides 4–7; population III – polypeptides 4, 5, 7; populations IV and V – polypeptides 4, 7 (Fig. 6). It could not be excluded that band 7 of population V was composed of more than one polypeptide. The amount of stainable material in band 4 in relation to the polypeptides in bands 1 and 2 was low in population I particles.
Fig. 7. Distribution of [3H]-arginine (○—○) and [14C]-threonine (●—●) after electrophoresis of polypeptides from virus particle populations I (incomplete virus, A) and V (intact virus particles, B) in neutral SDS containing polyacrylamide (7.5 %, w/v). Virus particles were isolated as described under the section on contamination of virus particles with host proteins.
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Table 3. Ratios of [3H]-arginine/[14C]-threonine in polypeptides from incomplete (I) and intact (V) type 16 adenovirus

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Incomplete virus*</th>
<th>Intact particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Band mol. wt.</td>
<td>(population I)</td>
<td>(population V)</td>
</tr>
<tr>
<td>1</td>
<td>124000</td>
<td>1.0†</td>
</tr>
<tr>
<td>2</td>
<td>79000</td>
<td>1.64 to 1.68</td>
</tr>
<tr>
<td>3+4</td>
<td>54000 to 60000</td>
<td>(0.94 to 1.16)§</td>
</tr>
<tr>
<td>5</td>
<td>37000</td>
<td>1.88 to 1.93</td>
</tr>
<tr>
<td>6</td>
<td>27500</td>
<td>1.16 to 1.31</td>
</tr>
<tr>
<td>7</td>
<td>26000</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>13000</td>
<td>(0.61 to 1.01)</td>
</tr>
<tr>
<td>9 &lt; 10000</td>
<td>—</td>
<td>(5.60 ± 0.24)</td>
</tr>
</tbody>
</table>

* Ratios obtained after separation of two and three different preparations of virus particle populations I and V, respectively. Each preparation was separately analysed by electrophoresis in SDS-polyacrylamide (7.5% w/v) gels.

† The ratios obtained for each polypeptide were divided by the ratio calculated for band 1 (hexons) in order to obtain a mean for comparison of different preparations of virus material.

‡ Standard deviation.

§ The arginine/threonine ratios for polypeptides 3+4, 8 and 9 presented within parentheses should be interpreted with caution since the amount of [14C]-threonine in these polypeptides was low.

Comparison of polypeptides characteristic for complete and incomplete virus by double labelling with [3H]-arginine and [14C]-threonine

Double labelling of adenovirus proteins with [3H]-arginine and [14C]-threonine was performed with the aim of identifying the different polypeptides that are characteristic for complete and incomplete virus (Fig. 7) by the relative content of arginine. The amount of [3H]-arginine was related to [14C]-threonine, since the amount of the latter amino acid varied within a restricted range (6.2 to 9.1 residues per 100) in different polypeptides of adenovirus types 2, 3 and 5 (Pettersson & Höglund, 1969; Laver, 1970; Pettersson, 1971; Prage & Pettersson, 1971; Russell, McIntosh & Skehel, 1971).

The distribution of radioactively labelled polypeptides obtained after electrophoresis (Fig. 7) agreed with one exception with the separation of stained polypeptides. After separation of complete particles, radioactivity, but no stainable material, was detected at the region of the tracking dye, indicating a polypeptide with a mol. wt. of less than 10000. Only slight amounts of this material were detected in virus particle population I. This polypeptide could correspond to peptide X of adenovirus type 2 (Maizel et al. 1968). Separations performed on different preparations of virus material were compared by dividing the ratios of the amounts of [3H]-arginine over [14C]-threonine obtained for each polypeptide with the ratio obtained for hexons (Table 3).

Two polypeptides which are characteristic for virus particle population I, 5 and 6, show a comparatively high (1.88 to 1.93) and a low (1.1 to 1.31) arginine/threonine ratio, respectively. Band 7 of intact virus, although being similar in size to band 6, is qualitatively different as indicated by the high arginine/threonine ratio (2.50), thus corresponding to the arginine-rich core proteins of adenovirus types 2, 3 and 5 (Laver, 1970; Prage & Petersson, 1971; Russell et al. 1971). It appears likely that in addition to the peak of radioactivity designed as band 7 a somewhat more rapidly migrating component occurs in intact virus and presumably also in virus particle populations II, III and IV (Fig. 6).
DISCUSSION

Preparations of adenovirus types 2, 12 (Burlingham & Doerfler, 1969) and 16 contain four, five and eight virus particle populations, respectively. The virus particle populations of type 16 display a pattern of buoyant densities which is reproducible upon analysis under standardized conditions. Degradation of complete particles is not considered to be a major source of the incomplete virus particles since their capsids appear morphologically intact as indicated by (a) electron microscopy on fresh preparations, (b) DNase resistance of DNA in incomplete virus particles, (c) the availability of type-specific but not group-specific antigenic determinants of the hexons, which form the virus capsid (unpublished observations). This indicates that the capsids of incomplete particles are intact since the surface of adenovirus particles is predominantly type-specific, whereas degraded adenovirus particles expose group-specific antigenic determinants (Norrby, Marusyk & Hammarskjöld, 1969). Furthermore, incomplete virus particles could not be detected after centrifuging virus on equilibrium density gradients. In addition, a distinct discrepancy between type 16 complete (population V) and incomplete virus particles (populations I to IV) is noted as concerns the frequency and occurrence of polypeptides with mol. wts. of 26,000 to 60,000. The difference in appearance of polypeptide band 5 between virus particle populations I and V of type 16 appear to correspond to the difference between polypeptides of complete particles and top components of types 3 and 7A (Maizel et al. 1968; Prage et al. 1972).

The mol. wts. of 79,000 obtained for polypeptides in band 2 is in agreement with the mol. wt. of 70,000 to 78,000 obtained for vertex capsomeres of types 2 and 5. The mol. wt. of fibre polypeptides of the latter types range between 60,000 and 69,000 (Maizel et al. 1968; Laver, 1970; Russell & Skehel, 1972). Under similar conditions of electrophoresis, polypeptides III and IV (according to the designation of Maizel) of type 7A did not seem to be separated (Maizel et al. 1968). This observation appears to agree with the observations on adenovirus type 16 in this study, consequently, it appears likely that a difference exists in mol. wts. of vertex capsomere or fibre peptides between types belonging to subgroup I (types 7A and 16) and III (types 2 and 5).

The difference between virus particle populations V (intact particles) and I (incomplete virus) as regards the ratio of arginine to threonine in the polypeptides of band 2 is somewhat difficult to explain. Assuming that the amounts of fibre + vertex capsomere polypeptides in complete and incomplete virus are the same, their relative content of still another polypeptide with a mol. wt. intermediate between vertex capsomere and fibre might differ. A polypeptide of this size has been demonstrated in particles of adenovirus type 2 (Laver, 1970), and also in studies of infected cell specific polypeptides (ICSP’s) by Russell & Skehel (1972). We have no information on the occurrence of ICSP’s in cells infected with adenovirus type 16 and it cannot be excluded that an ICSP, with a mol. wt. corresponding to that of vertex capsomere and fibre, could be assembled into incomplete virus particles.

Although no experimental evidence has indicated a precursor–product relationship between polypeptides of incomplete virus and those assembled into intact particles, the occurrence of different polypeptides in the four incomplete virus particle populations could correspond to precursor stages during development of an intact particle. The observed high arginine/threonine ratio in polypeptide band 5 (mol. wt. 37,000) of incomplete virus population I, and the even higher arginine/threonine ratio of virus polypeptide 7 (mol. wt. 26,000) corresponding to the arginine-rich polypeptide, which has been well documented in types 2, 3, 5 (Laver, 1970; Prage & Pettersson 1971; Russell et al. 1971), could be an indication of a precursor–product relationship.
Incomplete adenovirus

After the final step of separation of virus particle populations I to III, relatively small amounts of infectivity were found at the buoyant densities of complete particles, whereas the major part of the infectivity were found in peaks which were distributed corresponding to the haemagglutinins at the characteristic density of each virus particle population.

It was concluded that this was due to multiplicity-dependent infectivity of incomplete adenovirus particles. In a parallel study of incomplete particles of adenovirus type 3 it was demonstrated that the purified incomplete virus particle populations contained infectivity which was concluded to be caused either by aggregation of complete and incomplete virus particles or by complementation (Prage et al. 1972).

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