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PREPARATIVE SEPARATION OF INTACT INCOMPLETE AND EMPTY ADENOVIRUS TYPE 2 PARTICLES

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Summary. — Complete adenovirus type 2 virions containing the whole genome were resistant to while empty capsids lacking DNA were breakable at a separation producere involving customary CsCl gradient centrifugation. A purification method was developed using CsCl density gradient centrifugation in 5% glycerol which retained the integrity of incomplete particles as well as empty capsids. This procedure enabled to study the biological and physicochemical properties of different adenovirus type 2 particles.

Key words: *adenovirus*; *incomplete empty particles*; *gradient centrifugation*

Introduction

In productive adenovirus (Ad) infection several classes of particles are produced differing from mature virions (V) in CsCl buoyant density gradients. One class consists of empty (E) capsids lacking DNA (Maizel *et al.*, 1968; Prage *et al.*, 1972; Ishibashi and Maizel, 1974; Rosenwirth *et al.*, 1974), another which bands between virions and empty capsids, contains fragments of DNA (Prage *et al.*, 1972; Ishibashi and Maizel, 1974) and has been referred to as incomplete (IC) particles. Several different classes of incomplete particles varying in DNA content have been observed (Wadell *et al.*, 1973; Burlingham *et al.*, 1974; Rosenwirth *et al.*, 1974). In the case of Ad2, IC and E particles represent 5—15 per cent of the total amount of virions (Rosenwirth *et al.*, 1974). It has been suggested that incomplete and empty particles represent intermediates in the Ad assembly (Sundquist *et al.*, 1973; Ishibashi and Maizel, 1974; Daniell, 1976; Tibbets, 1977); this view was based mainly upon pulse-chase labelling experiments. The studies of IC and E particles may lead to further understanding of their various biological properties. The present study demonstrates that empty capsids may be fragile and do not resist to purification procedure involving CsCl gradient centrifugation and describes a method for preparative separation of IC and E particles preserving their integrity.

Materials and Methods

Cells and viruses. Human KB cells were propagated in suspension cultures using Eagle's medium supplemented with 7% calf serum. Human adenovirus type 2 (Ad 2) kindly provided by Dr. H. G. Pereira, National Institute for Medical Research, London, was replicated in KB cell suspension cultures. Plaque assay in HEp-2 cells was used for quantitation of the infectivity (Williams, 1970).

Preparative separation of the incomplete and empty particles of Ad 2. Cells infected with Ad 2 were pelleted by centrifugation, resuspended in 10mmol/l Tris-HCl buffer (pH8.1) and homogenized in a MSE sonicator. The suspension was extracted with equal volume of trichlorotrifluoroethane by agitation in a Sorvall omnimixer. Discontinuous density gradient was prepared by placing of 1/7 vol CsCl solution (density 1.50 g/ml) into a nitrocellulose tube and overlaying it with 1/5 vol CsCl (solution density of 1.20 g/ml). Both CsCl solutions contained 5% glycerol. Finally the tube was filled up with crude virus material carefuly pipetted onto the top of the gradient. The gradients were centrifuged for 3 hr at 25 000 rev/min in Beckman SW 27 rotor. The IC and E particles were separated in a second step density gradient composed of 3 CsCl layers (densities of 1.4, 1.3 and 1.2 g/ml) containing 5% glycerol by centrifugation at 25 000 rev/min for 12 hr in Beckman type SW 41 rotor. The fractions of both incomplete and empty particles were pooled and mixed with a CsCl solution (density of 1.32 g/ml) containing 5% glycerol for further fractionation. Equilibrium centrifugations in SW 41 rotor were performed twice on each virus preparation. The bottom of the tube was punctured and the collected fractions were dialysed against 5% glycerol in 10 mmol/l Tris-HCl and 1 mmol/l $MgCl_2$ at pH 8.1 and frozen at $-70°C$ as described by Edvardsson *et al.* (1978).

Electron microscopy. JEOLCO JEM type 100 B electron microscope was used. Purified virus samples were examined after negative staining with 2% uranyl acetate. To determine the distribution of different particles in the fractions obtained from the CsCl density gradient about 100 particles were counted and evaluated in each electron microscopic specimen.

Results

Purification of empty capsids by CsCl buoyant density gradient in the presence and absence of glycerol

The morphological appearance of empty particles separated by CsCl density gradient centrifugation was investigated. When neither the gradient nor the dialysis buffer contained glycerol, many collapsed and disrupted empty capsids were observed (Fig. 1a). In the presence of glycerol the preparative separation of intact empty particles was successful providing capsids of uniform morphology (Fig. 1b). The particles purified in the presence of glycerol were not aggregated. No damage of complete virions was observed due to the purification procedure regardless of the presence or absence of glycerol.

Characterization of complete, incomplete and empty particles

Fig. 2 (Plate XXV) shows the Ad2 particles centrifuged to equilibrium in CsCl density gradient containing 5% glycerol. The virus was fractionated as indicated in Fig. 2, to provide nine pools corresponding to density classes ranging from complete virions to empty capsids. The distribution of particles in CsCl gradient was not influenced by 5% glycerol, but it does not allow to measure the correct buoyant densities of fractions. The ratio of optical densities of each fraction measured at 260 and 280 nm together with their

Table 1. Characteristics of adenovirus particles fractionated by density gradient centrifugation

Fraction number*	OD_{260}/OD_{280}**	Infectivity PFU/ml	Specific infectivity*** PFU/OD_{280}
1	1.24	6.2×10^{10}	4.5×10^{10}
2	1.23	1.8×10^{10}	1.3×10^{10}
3	1.21	9.4×10^{9}	6.2×10^{9}
4	1.19	6.4×10^{9}	4.8×10^{9}
5	1.18	3.6×10^{9}	3.2×10^{9}
6	1.15	3.9×10^{9}	3.8×10^{9}
7	1.13	2.7×10^{9}	2.3×10^{9}
8	1.09	1.7×10^{9}	1.2×10^{9}
9	1.05	1.3×10^{9}	5.4×10^{8}

* The corresponding bands are shown on Fig. 2.
** All ratios were determined when $OD_{280} = 0.50$.
*** The specific infectivity was determined from the results of plaque assay in HEp-2 cells and adjusted to an $OD_{280} = 1.00$.

infectivity and specific infectivity values are shown in Table 1. The decreasing proportion of nucleic acid to protein in the particles was associated with decreasing of OD_{260}/OD_{280} ratio and infectivity. The specific infectivities of fractions determined from the results of plaque assay and adjusted to an $OD_{280} = 1.00$ showed a similar pattern of OD_{260}/OD_{280} ratios.

The particle morphology in each fraction was studied by electron microscopy using uranyl acetate as a negative stain. The distribution of particles of different morphology in the CsCl gradient is presented in Fig. 3. The particles were divied in two groups based on penetration of uranylacetate into capsids. First class (I) consists of particles which excluded the stain completely or almost completely. The second class (II) particles were intensively penetrated by the stain (Fig. 4).

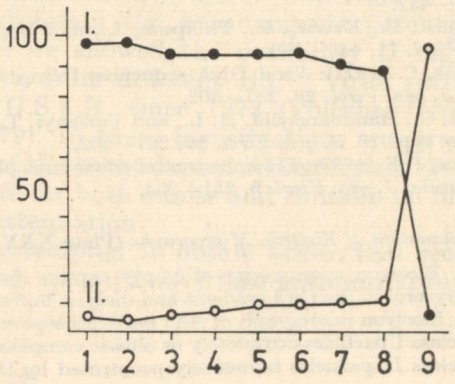

Fig. 3.

Distribution of Ad2 particles separated by CsCl density gradient centrifugation Class I particles: ●——●; class II particles ○——○. Electron micrographs of these particles can be seen in Fig. 4. Numbers of the corresponding bands are explained in Fig. 2.

Discussion

After purification of Ad2 in CsCl gradient many disrupted particles were present in the band of empty capsids (Fig. 1a) and several intact particles in the band of complete virions. This suggested that empty capsids were less stable than complete virions and did not survive without damage after prolonged centrifugation and dialysis. Using 5% glycerol in CsCl solution for virus purification and in Tris-HCl buffer for dialysis allowed to separate intact empty and incomplete particles. Nine individual virus pools from the range of complete virions to empty capsids were investigated by electron microscopy and other methods (infectivity, optical density). There was no close correlation between the morphological appearance of particles and their biological and physicochemical properties. The infectivity and the OD_{260}/ /OD_{280} ratio changed gradually throughout the gradient, whereas a rapid alteration in morphology was observed at the range of empty capsids (Figs 3 and 4).

The complete and highly infectious particles excluded the negative stain, while the incomplete particles were slightly and the empty capsids intensively wenetrated. It seems that modified purification procedure is useful to obtain ntact incomplete particles and empty capsids for the study of virus assembly.

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Explanation of Electron Micrographs (Plate XXV):

Fig. 1. Electron micrographs of empty capsids separated in the absence (a) and presence (b) of glycerol in the CsCl gradient and dialysis buffer. Length of the bar = 0.1 μm.

Fig. 4. Electron micrograph of Ad2 particles separated by CsCl density gradient centrifugation. I: class I particles completely or almost completely excluding the negative stain. II: class II particles intensively penetrated by the stain.