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PURIFICATION OF ADENOVIRUS HEXON PROTEIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Adenovirus (Type 2)-infected HeLa cells were sonicated and treated with 1,1,2-trichlorotrifluoroethane. The water-soluble extract was ultracentrifuged and the supernantant, containing the dissociated proteins, was subjected to anion-exchange high-performance liquid chromatography on a Mono Q column in 50 m*M* bis-Tris–HCl (pH 6.5). Elution with a linear salt gradient resulted in a major peak, containing the hexon protein.

INTRODUCTION

Adenoviruses are a family of non-enveloped icosahedral viruses of 41 human serotypes. The virus particle contains at least nine unique polypeptides of which hexon, penton and fiber are the major antigens associated with the structural proteins[1]. The hexon is the main component of the viral capsids and is produced in large excess by infected cells before lysis. Although there are variations between hexons of different serotypes[2], detection of the hexon antigen in clinical specimens is a sensitive means of adenovirus diagnosis[3].

Several methods in combination with conventional DEAE ion-exchange chromatography have been used for the isolation of the hexon protein for structural studies[4,5] and for antigen production[6]. In this study, high-performance liquid chromatography (HPLC) was applied to the purification of hexon protein for diagnostic purposes.

EXPERIMENTAL

Virus

Adenovirus, Type 2, was grown in HeLa-Ohio cells, maintained in Roux bottles with Eagle's minimal essential medium, supplemented with 2% foetal calf serum. Two days after inoculation, the cells showed fully developed viral cytopathic effect and were harvested in phosphate-buffered saline (PBS) with a rubber policeman. The cells were washed twice with PBS, once with 50 m*M* bis(2-hydroxyethyl)iminotris-(hydroxymethyl)methane–HCl (pH 6.5), (bis-Tris) (Sigma, St. Louis, MO, U.S.A.) and frozen at −70°C.

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Disruption of the cells

The cell pellet was thawed and diluted with two volumes of bis-Tris buffer. One volume of 1,1,2-trichlorotrifluoroethane (Freon, Merck, Darmstadt, F.R.G.) was added to the suspension. The cells were disrupted by 2–3 alternating cycles of vigorous mixing and sonication in a Branson B 15 sonifier (Branson Sonic Power, Darbyry, CT, U.S.A.) at 100% output for 30 s. The cell debris was removed by centrifugation at 1000 *g* for 30 min. The aqueous phase (upper layer) was collected and centrifuged for 30 min at 100 000 g. The supernatant was subjected to HPLC purification.

Anion-exchange chromatography

The purification of the hexon protein was performed on a Mono Q HR 10/10 column (100 × 10 mm I.D.) connected to a complete FPLC system (Pharmacia, Uppsala, Sweden). The sample was applied via a 10-ml superloop. Proteins were eluted with a linear salt gradient of 0–0.5 *M* sodium chloride in bis-Tris buffer. The gradient volume was 240 ml and the flow-rate was 4 ml/min. The eluate was monitored at 280 nm.

Analysis

The hexon peak from the column was identified and the recovery of the hexon protein was determined by time-resolved fluoroimmunoassay[7] (TR-FIA), as described elsewhere[8]. The assay was performed on a solid phase of polystyrene microstrip wells, coated with rabbit anti-hexon IgG, the bound antigen being detected by Eu-isothiocyanatephenyl-EDTA-labelled antibody (the same as on the solid phase). Owing to the sensitivity of the assay, the samples had to be diluted 10^5- or 10^6-fold. The protein concentration was determined with $A^{1\%}_{280\ nm} = 10.0$.

High-performance gel filtration was performed on a Superose 12 HR 10/30 column (100 × 30 mm I.D.) (Pharmacia) in bis-Tris buffer, containing 0.15 *M* sodium chloride, at a flow-rate of 0.4 ml/min. The column was calibrated with standard proteins (HMV calibration kit, Pharmacia). The void volume (V_0) was determined with viral nucleocapsids and the total volume (V_t) with acetone. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli[9] in 10% slab gels of 1.5 mm thickness and stained with Coomassie Brilliant Blue. The molecular weight markers for SDS-PAGE were supplied by Sigma. The gel was scanned with a Beckman Model CDS-200 densitometer (Beckman Instruments, Palo Alto, CA, U.S.A.).

RESULTS AND DISCUSSION

The viral proteins and intact virions were released from the cells by Freon extraction and sonication. Virions were pelleted by centrifugation and the supernatant, containing the dissociated proteins, was subjected to HPLC. By washing the cells with the bis-Tris buffer before extraction, the supernatant could be applied to the Mono Q chromatographic column without a buffer exchange.

The adenovirus hexon protein was eluted as a single, homogeneous peak just before the end of the gradient at *ca.* 0.45 *M* sodium chloride (Fig. 1). It was also the major peak, covering 10–20% of the total protein eluted from the column.

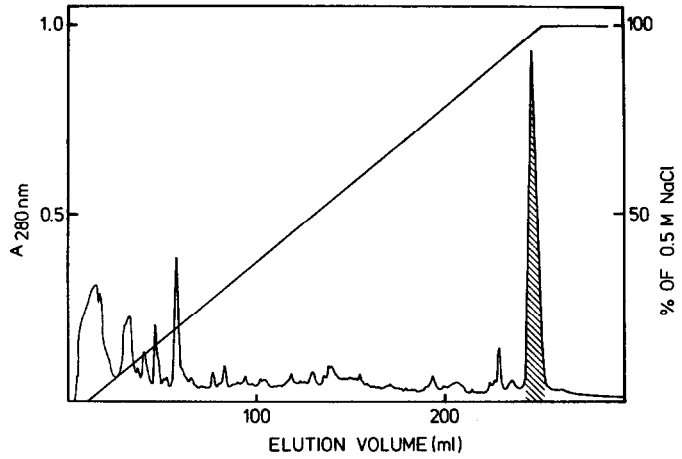

Fig. 1. Anion-exchange HPLC of soluble proteins in a adenovirus-infected HeLa cell extract. The Mono Q HR 10/10 columns was eluted with a linear salt gradient of 0–0.5 *M* sodium chloride in 50 m*M* bis-Tris–HCl (pH 6.5) at a flow-rate of 4 ml/min. The major peak (shaded), containing the hexon protein, was collected with a cut-off level of 0.05 absorbance unit.

A sample of the hexon fraction from the Mono Q column was analysed in native form by gel filtration on Superose 12 (exclusion limit 2 × 10^6 daltons). No additional peaks were found by monitoring the eluate at 280 nm (Fig. 2). With a cut-off level of 1% and a full scale of 1.0 absorbance unit, the integrated area of the hexon peak was 98% of the total monitored area. The K_{av} value of the hexon peak was 0.19, giving a molecular weight estimate of 235 000 when compared with standard proteins analysed under identical conditions (data not shown).

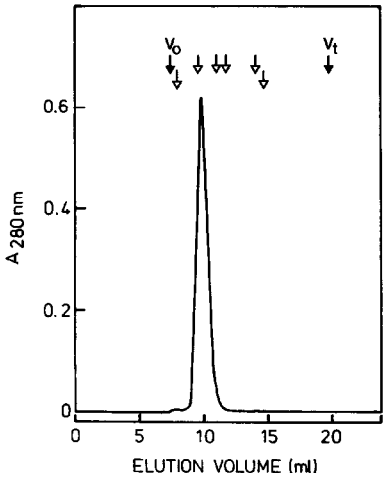

Fig. 2. High-performance gel filtration of the purified hexon protein. A 100-μl sample of the hexon peak from the HPLC on Mono Q was chromatographed on the Superose 12 HR 10/30 column with 50 m*M* bis-Tris–HCl (pH 6.5), containing 0.15 *M* sodium chloride, at a flow-rate of 0.4 ml/min. Standard proteins (open-headed arrows), from left to right: thyroglobulin (MW 669 000), ferritin (MW 440 000), aldolase (MW 158 000), albumin (MW 67 000), cytochrome *c* (MW 25 000) and ribonuclease A (MW 13 700).

The SDS-PAGE analysis occasionally revealed some faint contaminating bands or breakdown products when overloading amounts of the hexon preparation were applied (Fig. 3). The apparent homogeneity of the hexon band was 98.3%, measured by scanning of the gel. A subunit molecular weight of 110 000 was estimated from the SDS-PAGE; this agrees well with the subunit molecular weight calculated from the nucleotide sequence[10]. In the native form, the hexon consists of three identical polypeptide chains.

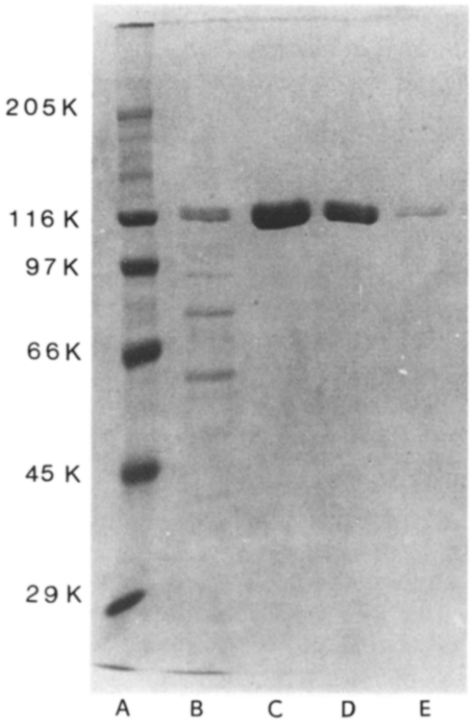

Fig. 3. SDS-PAGE analysis of the purified hexon protein. Lane A, molecular-weight markers (from top to bottom: myosin, *p*-galactosidase, phosphorylase b, bovine albumin, egg albumin, carbonic anhydrase); lane B, 10 μg of ultracentrifuged supernatant; lane C, 20 μg, lane D, 10 μg, and lane E, 1 μg of the hexon preparation.

In four experiments, the average yield of hexon protein was 4.1 mg from 20 Roux bottles. The recovery of immunologically active hexon was measured by TR-FIA (Table I) and averaged 89% (±9% S.D.) of the applied hexon in HPLC on Mono Q. No attempts were made to locate other soluble adenovirus proteins or "slow hexons"[4] in the chromatogram. However, if the Mono Q chromatogram is compared with that of slightly differently eluted DEAE-Sephadex A-50[4], a similar pattern of peaks is found with a better resolution and more minor peaks in HPLC.

As the capacity of the column was only partially used, it should be possible to increase the sample loads 5–10-fold without any loss of resolution of the hexon peak.

TABLE I

RECOVERY OF PROTEIN AND IMMUNOREACTIVE HEXON DURING PURIFICATION

Average values of four experiments.

Fraction	*Protein (mg) (mean ± S.D.)*	*TR-FIA response (%) (mean ± S.D.)*
Sonicated freon extract	70 ± 17	100
Ultracentrifuged supernatant	35 ± 11	45 ± 6
Mono Q hexon peak	4.1 ± 0.6	40 ± 8

The hexon preparation, bound to the solid phase on microtitre wells, was used successfully to capture human adenovirus antibodies of convalescent sera in enzyme immunoassay[11].

The results indicate that HPLC is a fast and simple technique for purifying adenovirus hexon protein in high yields. We are attempting to purify hexons of different adenovirus types with the same method for immunological comparisons.

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