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Improved high-performance liquid chromatographic method in the analysis of adenovirus particles

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Abstract

We developed a HPLC method on a novel continuous bed matrix (UNO Q, Bio-Rad) for the direct quantification of adenoviral type 5 (Ad5) particles produced in 293S Human Embryonic Kidney cells and compared this with an existing HPLC method on a conventional ion-exchange resin (Resource Q, Pharmacia). The 293S cell extract contained large amounts of DNA. This contaminated the viral peak on the Resource Q column and only after Benzonase treatment was it possible to quantify the viral particles in the cell extract. In contrast, the virus peak on the UNO Q column was resolved from the DNA which eliminates the need for pretreatment of the sample with Benzonase. Cross-analysis of the Ad5 fraction from the UNO Q column using a size-exclusion HPLC column revealed no additional contaminating peaks. We conclude that the purity of the Ad5 virus peak on the continuous bed matrix UNO Q column was superior to the purity of the virus on the conventional Resource Q column, which is essential for reliable quantification. Published by Elsevier Science B.V.

Keywords: Adenovirus particles; High-performance liquid chromatography analysis

1. Introduction

Adenovirus type 5 is a non-enveloped virus which has a particle size of approximately 80 nm and a molecular mass of about 170 000 000 [1]. The ability of easy penetration of adenoviruses into the cell makes it very attractive for gene transfer, such as for cancer gene therapy [2,3]. Gene transfer experiments require large amounts of highly pure viral material. Concerning the large-scale purification of adenoviruses, there is a need to shift from CsCl centrifugation [4] towards ion-exchange, hydrophobic interaction, metal chelate and size-exclusion chromatography [5,6]. The increased demand of pure virus preparations and the rapid development of new techniques necessitate new and fast quantification methods for the Ad5 virus. A biological analysis of viral activity is still cumbersome and time consuming. Usually a plaque assay takes 1–2 weeks to perform and the error in the final result can be more than 50% [7]. Fluorescent labeling and microscope tracking of adenoviruses was proposed and opened new possibilities for direct analysis of viruses [8].

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The green fluorescent protein (GFP) can be used as a marker instead of the β-galactosidase (LacZ) protein for initial development of viral vectors [9]. By expression of GFP in the cells the quantitative analysis of viral activity can be estimated by cyto-fluorimetric methods [10–12].

More recently, Shabram et al. used anion-exchange high-performance liquid chromatography (HPLC) after Benzonase treatment to detect intact particles [13], while the protein subunits of the viral particles have been separated by reversed-phase HPLC [14].

A new principle in producing chromatographic media – continuous bed chromatography – was announced recently for the purification of recombinant proteins [15]. With the continuous bed principle, the polymerization of the gel occurs directly in the column and creates a support derivatized with either the strongly basic quaternary amine group (UNO Q) or the strongly acidic sulfonic group (UNO S). The completely homogeneous channeling matrix results in an improved active mass transfer and a minimization of band broadening thus resulting in an increase in selectivity and resolution.

We decided to use the increased selectivity and resolution of the anionic continuous bed material to detect and quantify the Ad5 particles in the monitoring of various productions and further purification of Ad5 viruses for gene transfer experiments.

Part of this work has been presented at the Williamsburg Conference. Viral Vectors & Vaccines. Williamsburg, VA, 16–19 November 1998.

2. Materials and methods

2.1. Cell culture

The 293S cells were maintained in suspension cultures in a custom-made calcium free Dulbecco's Modified Eagle medium (CFDMEM; American Bioorganic, Niagara Falls, NY, USA) supplemented with 5% (v/v) COSMIC bovine calf serum (BCS; Hyclone, Logan, UT, USA) and 0.1% (w/v) Pluronic F68 (Gibco, Grand Island, NY, USA) as described previously by Nadeau et al. [16].

293SF-3F6 (293SF), a clone derived from the 293S and growing in suspension and serum-free medium was also used for bioreactor runs. In this case, a low-calcium serum-free (LC-SFM), as described in Côté et al. [17], was used for these experiments.

Adenovirus type 5 containing the GFP under the control of the CMV promoter (Ad5 GFPq) was kindly provided by Bernard Massie, Biotechnology Research Institute, Montreal, Canada. The viral stock titer was $7.5 \cdot 10^{12}$ pfu/ml.

The fed-batch culture was operated as described in Côté et al. [17], Briefly, virus stock in 100 ml fresh LC-SFM medium was added to cell culture at $1 \cdot 10^{6}$ cells/ml in a 3-l Chemap CF2000 bioreactor (Mannedorf, Switzerland). The temperature is maintained at 37°C. The pH was controlled at 7.1 by feeding an air–CO_2 mixture and 1 *M* NaOH and dissolved oxygen (DO) was controlled at 30%. On-line data acquisition and control were performed with the FIXMMI software (Intellution, Norwood, MA, USA). A fed-batch was started right after virus addition with a flow-rate of 0.01 ml/min increasing to a final flow-rate of 0.06 ml/min. The mixture added was composed of 150 ml MEM amino acids, 25 ml glucose at 300 g/l and 50 ml of 400 m*M* glutamine.

2.2. Cell lysate preparation

The 293S or 293SF cells in culture media were centrifuged (Sorvall RC 3B Plus) 2–10 min (depending on the sample volume) at 395 *g*. The supernatant was removed and the cells were resuspended in a low-ionic-strength buffer to 10 times less the original volume and frozen at −80°C until needed. Prior to analysis, the samples were thawed at 37°C. After the third freeze–thaw cycle, the samples were clarified by centrifugation at 700 *g*, filtered through a 0.22- or a 0.45-μm syringe filter and the supernatants were transferred to 250-μl glass vials for injection. In other samples, cellular and viral DNA was removed by adding Benzonase (Ultra Pure grade; EM Industries) 100 units enzyme/ml of lysate. The samples were gently mixed for 30 min at room temperature and were clarified as above. Storage of the lysate supernatants at room temperature for more than 1 h

caused the samples to become nebulous and more viscous. Longer storage, even at 4°C, resulted in the formation of a sediment inside the vials, especially in those lysates concentrated more than 10-fold.

2.3. Freeze–thaw technique

Infected 293S cells were harvested by centrifugation, resuspended in buffer and frozen at −80°C for at least 2 h. Thereafter, the frozen cell suspension was thawed at room temperature and a sample of the extract was then centrifuged and applied unto the UNO Q-1 column for quantification of the total viral particles. An infectivity test was also performed on the same samples. This procedure was repeated a total of 10 times.

In a parallel experiment, after the first freeze–thaw cycle, cellular debris was removed by centrifugation and the supernatant was filtered through a 0.45-μm filter. The bulk of the filtered supernatant was then subjected to another nine cycles of freeze–thaw with samples taken after each cycle to be analyzed in the same way as the first series.

2.4. Infectivity assay

The recombinant adenovirus produces the marker protein GFPq which has an excitation and emission wavelength of 490 and 510 nm as well as increased emission signal intensity detectable in single cells by either fluorescent microscopy or flow cytofluorimetry – fluoresence activated cell sorting (FACS) [18]. A biological method was used to measure the number of infectious viral particles (IVPs) in a sample by taking into account virus penetration as well as a viral gene expression. 293S cells were maintained in exponential growth phase then centrifuged and resuspended in fresh medium prior to infection. Infections were carried out in six-well plate at $1 \cdot 10^6$ cells/ml at 1 ml/well. A multiplicity of infection (MOI) of 2, 4, 6, 8 and 10 where used for the reference wells and different dilutions for the unknowns. After incubation at 37°C with shaking in a 5% CO_2 atmosphere, the cells were harvested between 18 and 22 h post infection (hpi). The cells were then resuspended in 0.5 ml of phosphate-buffered saline (PBS) to which 0.5 ml of a 4% formaldehyde solution was added drop by drop under constant gentle agitation to prevent cell aggregation. This mixture was incubated at 4°C for a minimum of 30 min to fix the cells. The cell suspension was then filtered through a 60-μm screen mesh and assayed by FACS. Green fluorescence of the GFPq was detected using a Coulter flow cytometer EPICS XL-MCL with a 525-nm band-pass filter (Coulter, Miami, FL, USA).

2.5. Total viral particle analysis

Anion-exchange HPLC analysis was performed on a Resource Q, 1 ml column, 3 cm×0.64 cm I.D. (Pharmacia Biotech, Piscataway, NJ, USA). Continuous bed column chromatography was performed on a UNO Q-1 (3.5 cm×0.7 cm I.D.) or a UNO Q polishing column (1 cm×0.46 cm I.D.) (Bio-Rad Labs., Hercules, CA, USA). Size-exclusion HPLC was performed on a TSK G 6000 PW_{XL} (30 cm×0.8 cm I.D.) column (Tosohaas, Montgomeryville, PA, USA). The Hewlett-Packard 1090 HPLC system with a photo diode array detector was used to monitor UV absorption. One of the parameters for the determination of the purity of the Ad5 peak is the ratio of the peak area or peak height at 260 nm/280 nm= 1.2–1.3. The UV absorption was set up at 260 and 280 nm for the detection of DNA, Ad5, and proteins and at 470 nm for the detection of GFP. The buffer consisted of 5 m*M* 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) (pH 7.5) with an increasing NaCl gradient for the anion-exchange (AE) HPLC (see Fig. 1a), and 50 m*M* Tris–HCl (pH 7.5) for the size-exclusion (SE) HPLC. The flow-rate for both AE- and SE-HPLC was 1 ml/min.

Since the cell lysate samples are quite heterogeneous and precipitation may occur, we found that we increased the lifetime of the column by adding a pre-column filter housing containing a 0.5 μm frit.

2.6. Preparation of the Ad5 standard

Our Ad5 standard was purified by a two-step CsCl ultracentrifugation procedure and the particle number was defined by measuring the absorbancy at 260 nm

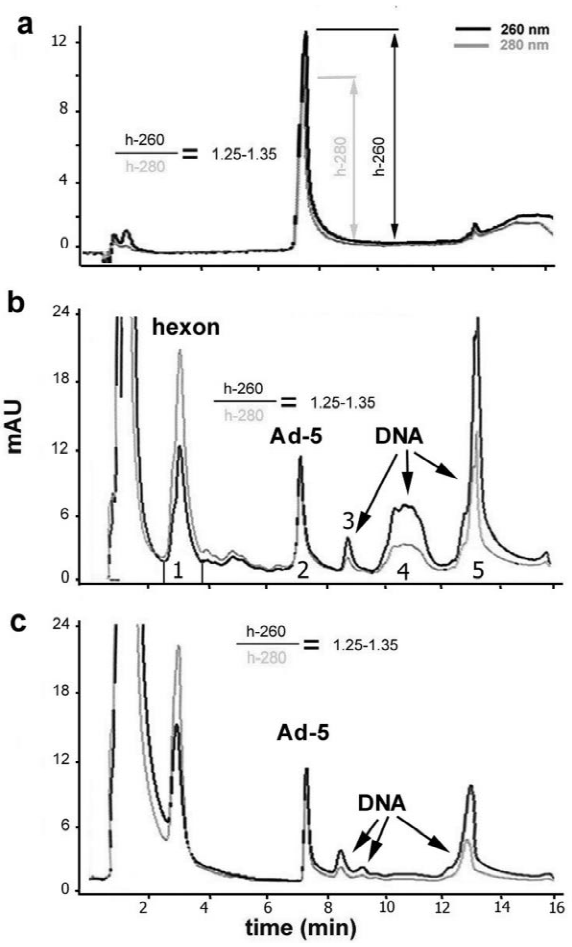

Fig. 1. The separation of Ad5 particles by anion-exchange HPLC on a continuous bed UNO Q column. A 20-μl volume of a sample was injected on the column and eluted with an increasing NaCl gradient (as indicated) at a flow-rate of 1.0 ml/min. The black line shows the absorption at 260 nm, while the grey line depicts the absorption at 260 nm. h-260 and h-280 is the UV absorption of the peak at 260 and 280 nm, respectively. The h-260/h-280 ratio is used as a measure of the purity of the viral peak. (a) An Ad5 standard, purified by ultracentrifugation on a $CsCl_2$ gradient. (b) 293S cell extract, before treatment with Benzonase. (c) 293S cell extract, after treatment with 100 units of Benzonase/ml of cell extract for 30 min at room temperature.

in the presence of SDS as described by Shabram et al. [13]. The purity of the standard was confirmed by AE-HPLC, both with the Resource Q and UNO Q columns, and with SE-HPLC. The purified standard was diluted in storage buffer, aliquoted and stored at −80°C.

3. Results

3.1. Comparison of separation by UNO Q and Resource Q columns

3.1.1. UNO Q-1 continuous bed anion-exchange column

Cell extracts were analyzed on the UNO Q-1 column (Fig. 1). After obtaining a commercially available column, we changed the polymeric frits on both sides of the column to a layer of 10 nylon screens (200 μm pore size), because we found that the standard frits absorbed some Ad5 viruses. The samples were run at a flow-rate of 1.0 ml/min in a 5 m*M* HEPES (pH 7.5) buffer with an increasing NaCl gradient as indicated in Fig. 1a. A 20-μl injection of the purified Ad5 standard was eluted as a single peak (Fig. 1a), with a 260/280 nm absorption ratio ($A_{260/280}$) of 1.25–1.35.

The chromatogram in Fig. 1b shows the results when 20 μl of a clarified Ad5 virus extract from 293S cells were injected on the UNO Q column. The 293S cell extract was separated into several peaks. Peak 1 had a $A_{260/280}$ ratio of about 0.52 and a UV spectrum typical of proteins, peak 2 had a $A_{260/280}$ ratio of 1.25–1.35 with the same retention time and UV spectrum as the Ad5 virus standard. Peaks 3–5 had an $A_{260/280}$ of about 2 and the UV spectrum typical of DNA.

Fig. 1c shows the chromatogram of the same extract after Benzonase treatment (100 units/ml extract, shaken slowly at room temperature for 30 min). After clarification by centrifugation, 20 μl were injected onto the column. After Benzonase treatment of the same cell extract, the Ad5 viral peak area, height and $A_{260/280}$ ratio was unchanged, but although the total DNA peaks were reduced dramatically, they were not totally removed (Fig. 1c). We conclude that with the UNO-Q column, the Ad5 virus is well separated from the DNA peaks and therefore this method can be used for direct quantification of Ad5 particles in extracts from 293S cells,

without any prior DNA removal by Benzonase treatment.

3.2. Resource Q anion-exchange column

We evaluated the HPLC method for the detection of Ad5 viruses on a conventional Resource Q anion-exchange column, as published by Shabram et al. [13]. The chromatogram of 20 μl of a pure standard Ad5 solution on this column is shown in Fig. 2a and shows 1 major peak was detected. The $A_{260/280}$ ratio was 1.25–1.3, which is typical for a pure Ad5 preparation.

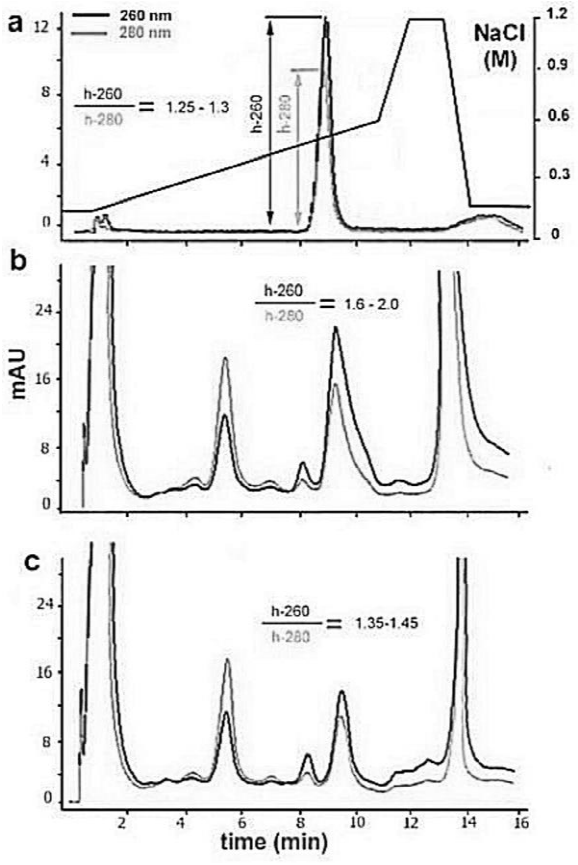

Fig. 2. The separation of Ad5 particles by anion-exchange HPLC on a Resource Q column. A 20-μl volume of a sample was injected on the column and eluted with an increasing NaCl gradient at a flow-rate of 1.0 ml/min. The chromatographic conditions and the samples were similar to those in Fig. 1. (a) An Ad5 standard, purified by ultracentrifugation on a $CsCl_2$ gradient. (b) 293S cell extract, before treatment with Benzonase. (c) 293S cell extract, after treatment with 100 units of Benzonase/ml of cell extract for 30 min at room temperature.

The chromatogram in Fig. 2b shows the results when 20 μl of a clarified Ad5 virus extract from 293S cells were injected onto the Resource Q column. Several peaks were detected. The second major peak has an $A_{260/280}$ ratio of about 0.5, which is typical of proteins. The third major peak had a $A_{260/280}$ of 1.6–2.0, and has the same retention time as the pure virus standard. The high $A_{260/280}$ ratio indicated that the Ad5 could be contaminated with DNA. The $A_{260/280}$ ratio varied per extraction preparation and the estimated DNA could range from 20 to 95%. The last peak in the chromatogram consists of DNA, which elutes from the column at a high salt concentration.

Fig. 2c shows the chromatogram of the same extract after Benzonase treatment. The chromatogram is comparable to the results obtained by Shabram et al. [13]. The DNA-contaminated Ad5 peak area was reduced, as compared with Fig. 2b and the $A_{260/280}$ ratio was lowered to 1.35–1.45 – a good indication that most of the contaminating DNA has been removed. However, the small increase in the $A_{260/280}$ ratio as compared with the standard (Fig. 2a) could indicate a small amount of residual DNA. The stripping of residual DNA from viral particles is a general problem [19], and even after Benzonase treatment a significant amount of residual DNA may remain in the preparation [20].

3.3. Size-exclusion chromatography

To verify the size distribution and the purity of the various fractions from the UNO Q-1 column, we collected the fractions from the UNO Q column as defined in Fig. 1b and reanalyzed these fractions by SE-HPLC on a TSK G 6000 PW_{XL} column. The column was calibrated with the CsCl purified Ad5 standard (which eluted near the exclusion limit), bovine serum albumin (66 000) and sodium chloride (58 000). Fraction 1 from the UNO Q-1 column (see Fig. 1b), consisted of one peak on the SE-HPLC column with a molecular mass of about 330 000 (Fig. 3a). This is in agreement with the molecular

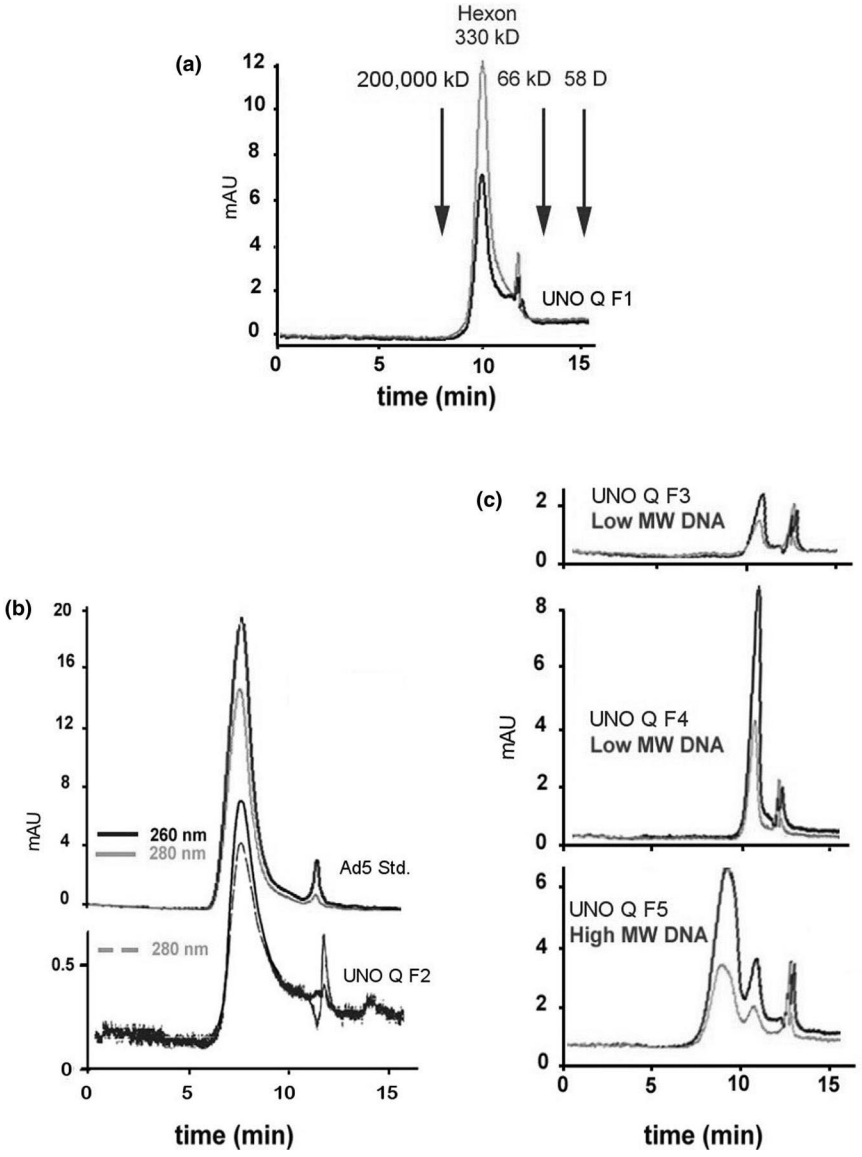

Fig. 3. Cross-analysis of the UNO Q eluted fractions by size-exclusion HPLC. A 100-μl volume of a 293S cell extract containing adenoviruses, was injected on a UNO Q column. Several fractions of the eluate of this column were further analyzed on a TSK G 6000 PWXL column. The collected fractions are indicated in Fig. 1b. (a) Protein fraction 1. The eluted peak has an apparent molecular mass of about 300 000, which is comparable with the molecular mass of the viral hexon trimer. (b) Fraction 2. The eluted peaks are in the void volume of the size-exclusion column. The top panel is a pure Ad5 standard solution (see Fig. 1a), while the bottom panel represents the adenovirus fraction 2 from the 293S cells. (c) DNA fractions 3, 4 and 5.

mass of the hexon trimer, the major structural unit of the Ad5 [21]. The SE-HPLC analysis of fraction 2 from the UNO Q-1 column showed one peak with a retention time similar to the Ad5 standard (Fig. 3b, bottom and top chromatogram, respectively). Fractions 3 and 4 collected from the UNO Q-1 column,

consisted mainly of relatively low-molecular-mass DNA, while fraction 5 contained mainly high-molecular-mass DNA (Fig. 3c).

With these SE-HPLC analyses we have confirmed that the Ad5 peak eluting from the UNO Q column is free of of hexons and major DNA contaminants and is of comparable purity to the Ad5 standard obtained from the CsCl density ultracentrifugation procedure.

3.4. Total viral particle quantification using the UNO Q column

In order to validate the analysis of Ad5 by AE-HPLC with the UNO Q column we evaluated the linearity of calibration plot, the injection stability and optimal storage conditions for the viral standard using both a UNO Q-1 column and a UNO Q polishing column.

Ad5 total particle numbers were calculated as described by Shabram et al. [13]. After estimation of particle numbers in the standard by absorbance at 260 nm in the presence of SDS, different volumes of the same concentration or different dilutions of the same standard were injected onto the columns. In both cases the plot exhibited a linear dependence of peak area versus the number of injected viral particles (Fig. 4). Standard curves were typically constructed by injecting 3–5 times the Ad5 standard samples with amounts ranging between $4 \cdot 10^{7}$–10^{11} total particles. The injection stability was checked over a period of several weeks by repeatedly injecting 20 μl of the CsCl purified Ad5 standard solution with a concentration $2 \cdot 10^{11}$ viral particles/ml. The standard deviation of the peak area was not higher than 5–10%.

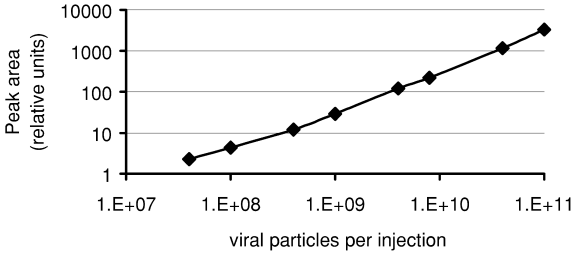

Fig. 4. Calibration curve of Ad5 total virus particles by HPLC on a UNO Q anion-exchange column. A 20–200-μl volume of the Ad5 standard solution at various dilutions was injected onto the UNO Q column. The absorption of the Ad5 peak at 260 nm was measured and integrated. The concentration of the $CsCl_2$ purified Ad5 standard solution was measured in the presence of SDS and calculated according to Shabram et al. [13], as mentioned in the Materials and methods section.

3.5. Application of the AE-HPLC method in the cell lysis step

As was mentioned previously, by using the UNO Q column we could inject clarified cell extracts directly, without Benzonase pretreatment. This direct procedure was used to investigate the cell lysis step on viral recovery and stability. We harvested infected 293S cells as per the freeze–thaw procedure in Section 2.3. The results are plotted in Fig. 5. We did not observe an increase in the Ad5 concentration during the first three freeze–thaw steps of the cell extract. In contrast, we noticed that after each additional step in the freeze–thaw procedure the concentration of the virus particles decreased as well as its capacity to infect new cells.

In the parallel experiment with the cell extract supernatant, we centrifuged then filtered the superna-

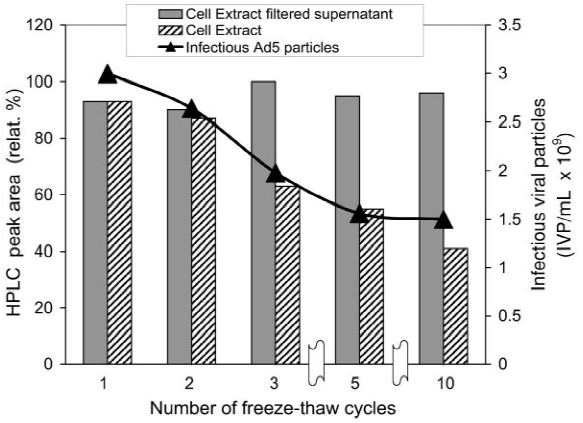

Fig. 5. Ad5 levels during repeated freeze–thaw cycles of an infected 293S cell extract and cell extract supernatant. An infected 293S cell suspension in spent media was frozen at −80°C then thawed at 37°C. Part of the extract was frozen and thawed nine more times. Another part was clarified by centrifugation and the supernatant filtered through a Millipore 0.45-μm filter. The filtered supernatant was frozen and thawed nine more times. △___△ depicts the amount of infectious virus particles of the unclarified cell extract.

tant through a 0.45 μm filter after the first freeze–thaw step. Now the results show that the concentration of total viral particles was stable during the successive freeze–thaw cycles of the filtered supernatant (Fig. 5). Data from the infectivity test (not shown) imply that there is no loss of infectivity as well.

Most probably the decrease in the concentration occurred because of the interaction of the virus particles with the cellular debris and/or an aggregation of the particles which were eliminated once we clarified the extract before injecting on the column. One cycle of the freeze–thaw procedure appears to be sufficient for the release of the viral particles and should be followed immediately with a clarification step.

3.6. Application of the AE-HPLC method in the monitoring of cell cultivation processes

We tested our method by monitoring the production of Ad5 in 293SF cells. 293SF cells were grown at $1 \cdot 10^6$ cells/ml and infected by the Ad5 GFPq. A fed-batch of amino acids, glucose and glutamine was started to feed critical nutrients during the infection phase [17,22]. At regular intervals, samples were drawn from the bioreactor and frozen at −80°C. Total viral particles produced was measured by our UNO Q HPLC method, as well as the GFPq expression by the Ad5. The production of the GFPq can be detected by the same system as for total viral particles. By scanning at 470 nm and changing the NaCl gradient we can define the GFPq as a separate peak and quantify its expression during the cell culture production together with the Ad5 (Fig. 6). The GFPq is the only substance which absorbs at 470 nm and can be easily detected as a single peak. In Fig. 7 we have plotted the results of an Ad5 GFPq production in 293SF cells. Time zero corresponds to the infection of the cells. It appears that the expression of the GFPq follows an exponential trend after 10 hpi, peaks at approximately 35 hpi and then slowly declines. This is consistent with our observations of GFPq using an on-line monitoring probe.

Virus production, as detected by our UNO Q HPLC method, appeared after 15–20 hpi, peaks at approximately 35 hpi and starts to decline thereafter. At 70 hpi, we have lost half of the total viral production. Viable Ad5 particles, as measured by the FACS method, followed the same growth kinetics, although at a lower percentage to total viral particles. The concentration of viable viral particles declines slower than that of the total viral particles, most probably because of the inherent stability of the complete, active particle. The ratio of infectious versus total viral particles varies depending on the production. This is in agreement with the finding of others [5,13].

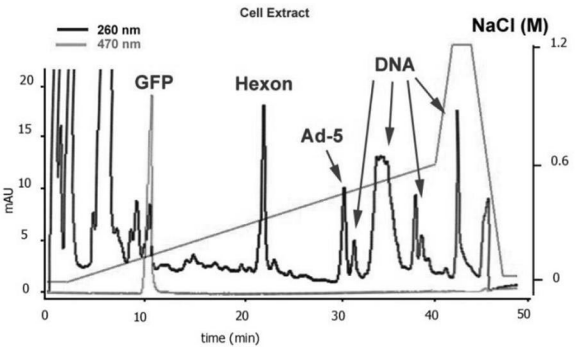

Fig. 6. Analysis of Ad5 containing 293S cell extract on a UNO Q column. A 50-μl of 293S cell extract was injected on a UNO Q polishing column and eluted with a shallow NaCl gradient as indicated. The black line indicates the UV detection at 260 nm and shows the protein, DNA and Ad5 peaks; the gray line indicates detection at 470 nm and shows the emission signal for the GFPq.

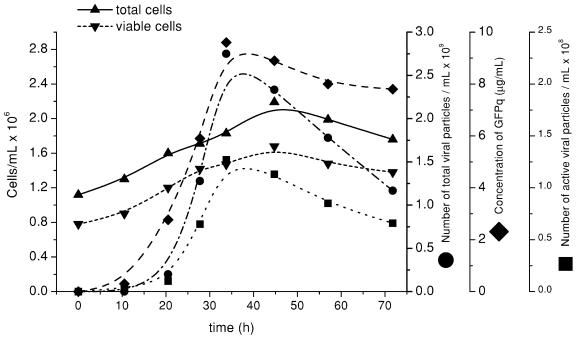

Fig. 7. Monitoring the Ad5 virus and other major parameters in a 3 l production of 293SF cells. 293SF cells were grown in a 3 l bioreactor and were infected with Ad5 viruses at time 0 min as described by Côté et al. [17]. At specific time intervals, 1 ml of the medium (containing 293SF cells) were collected and analyzed.

4. Discussion

To monitor viral production and the subsequent purification procedure, rapid and reliable viral detection methods are needed. HPLC methods for the detection of Ad5 viruses are rapid and can be automated, however, the Ad5 virus peak needs to be pure and free of contaminants. Unlike the classical chromatographic bed filled with spherical beads (Resource Q), the UNO Q column material from Bio-Rad consists of an in-column polymerized continuous porous matrix containing channels. This results in a high number of channels and a bigger surface area resulting in better mass transfer, which is essential for larger molecules such as viral particles [15]. Indeed we found this to be true in the separation of Ad5 particles on the UNO Q column. Thanks to the enhanced separation power of this column we were able to separate Ad5 viral particles from the free hexons, pentons, fibers and other proteinaceous material as well as DNA in the 293S cell extracts and therefore this newly developed method can be used for the detection and quantification of the Ad5 viruses during cell culture productions.

It should be noted, that the continuous bed columns are more sensitive to fouling. This is generally seen as an increase in pressure and a decrease in resolution. It is evident that the injection of lysates will foul the column much quicker than the injection of the Ad5 virus in a buffer. In this laboratory, the UNO Q column can be used for a few months or more depending on the nature of the sample to be injected and a stringent cleaning procedure is adopted after every run. Also, all samples should be filtered through a 0.22- or a 0.45-μm syringe filter prior to injection to remove possible viral aggregates and other precipitates. We did encounter some loss of the viruses on the column. This was mainly due to the frits situated at both ends of the column, which consisted of a porous polymer. Replacement of these frits with a layer of nylon screens resolved this problem.

As has been reported by others [5], the HPLC method only measures total particles and cannot distinguish between infectious and non-infectious particles. However, in our study of a 3 l batch production of Ad5 particles in 293SF cells, both the increase of total particles and the increase of infectious particles followed a similar pattern between 10 and 35 hpi. The appearance of the GFPq, as a marker for viral production in the cells and medium, was earlier than the virus measurements but again followed the same pattern. Because there seems to be a strong association between the Ad5 levels (whether total or viable particles) and the GFP measurements, we conclude that this improved HPLC method can be used to evaluate the optimal conditions for viral production. We have already seen its usefulness in maximizing the cell lysis step. From our results we saw that the freeze–thaw cycle itself is not detrimental to the stability of the virus.

5. Conclusions

In conclusion, we describe here a novel quantitative HPLC method for Ad5 viruses on a continuous bed column (UNO Q column) from Bio-Rad. Our results demonstrate that this matrix is able to efficiently separate Ad5 from contaminating proteins and DNA. Pretreatment of the cell extract samples with Benzonase is not required. This method is linear up to 10^{12} particles and has been tested under different conditions. This method has been proven useful for monitoring Ad5 viruses in cell culture productions and in the subsequent purification procedure.

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